USE OF SIMPLE, INEXPENSIVE MICROBIAL WATER QUALITY TESTS

RESULTS OF A THREE-CONTINENT, EIGHT- COUNTRY RESEARCH PROJECT

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THE USE OF SIMPLE, INEXPENSIVE MICROBIAL WATER QUALITY TESTS

Results of a three-continent, eight-country research project

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FOREWORD

Conventional bacteriological tests for assessing water quality are too expensive and impractical for routine use in the vast majority of developing countries. As a result, drinking water sources in most rural areas of the world often remain unevaluated.

For the past six years, the International Development Research Centre (IDRC) has supported research in the development of simpler and cheaper tests that would permit the monitoring and classification of drinking water sources. Researchers in Brazil, Chile, Egypt, Malaysia, Morocco, Peru, Singapore and Thailand have examined a number of promising non-traditional microbiological tests and have adapted them to their particular conditions and needs. Correlation/relationship studies between these simplified tests and the conventional tests such as total coliform, faecal coliform and *E. coli* were conducted. In September 1988, a global end-of-project meeting marking the end of the initial laboratory research phase was held in Banff, Alberta. This document has been prepared from the presentations and discussions of the meeting. It also includes a listing of previously published papers in scientific journals that have originated from this network of projects. It is our hope that this publication will consolidate and disseminate the findings of this global research effort.

Alex Redekopp
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REVIEW OF THE IDRC PROJECT TO EVALUATE FOUR SIMPLE, INEXPENSIVE, MICROBIAL WATER QUALITY TESTING PROCEDURES

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ABSTRACT

This review describes the development of the eight country, three continent, International Development Research Centre (IDRC) project for evaluating simple, inexpensive microbiological water testing procedures. The tests evaluated and compared were the coliphage test, A-1 broth (MPN) test and the P/A and H_2S paper strip potable water tests. A summary of the observations and conclusions of these studies is presented.

INTRODUCTION

In keeping with the goals of the United Nation's Water Decade Program, developing countries are concentrating on providing adequate quantities of drinking water to their populations. Unfortunately, little attention is being given to protecting and monitoring water quality. If this trend continues, the long-term effect would be a continued high incidence of water-borne diseases.

It is accepted that the primary purpose of water supply programs is to deliver potable water which is safe, adequate and accessible to all. However, in developing countries this task is not easy. Limited resources must be directed towards achieving an optimum balance between all three objectives. Moreover, when a water source is developed, it must be maintained. Water systems which fall into disrepair because of the lack of maintenance, waste scarce resources and defeat the purpose of the program.

Important considerations in the development and maintenance of safe water supplies are the setting of realistic standards and use of appropriate monitoring technology for assessing bacteriological water quality. In the developed and developing countries there are no simple solutions to the above considerations. Realizing that it is nearly impossible for rural water resources to meet established standards, ministries of health tend to allocate too few resources to water quality control programs.

However, if a classification scheme could be established based on microbiological properties, then these drinking water sources could at least be categorized and prioritized according to the levels of contamination or perceived relative health risk and perhaps the degree of sanitary protection available or used for the source waters. To implement such a scheme would require the monitoring of all potable water sources and the potable waters on a routine basis. One of the immediate impediments of this goal is the lack of appropriate inexpensive simple technology and laboratory facilities.

Bacteriological water quality tests presently being used have several disadvantages for routine use in developing countries. Firstly, they are not easily portable for use in rural areas. They require either trained technicians, sophisticated laboratory equipment or expensive supplies, most of which are not readily available in developing countries. In some instances, the long incubation time required for some tests before results can be obtained is a hindrance. These limitations seriously inhibit the effectiveness of most water quality control programs.

To overcome these problems, simplified, inexpensive, reliable microbiological water quality tests are required. In some instances, rapidity of results would be of immense benefit, if this could be achieved economically. Testing procedures which fulfil the above requirement should make it possible for countries to monitor and classify their raw water supplies and potable waters with a minimum of input in terms of resources and expertise. Consequently, priorities and goals could be established for maintaining and improving drinking water quality, thereby protecting public health.

In 1983, the International Development Research Centre (IDRC)-sponsored project identification seminar was held in Singapore to discuss the problems and the options for developing such tests. From this seminar emerged the concept and protocols for an IDRC-funded eight country, three continent research study on the feasibility of using several nontraditional microbiological procedures for estimating water quality and using these data along with sanitary surveys of the raw and potable water sources to characterize and categorize these waters.

Initially the project goals were to evaluate the use of a bacteriophage commonly called coliphage, and an enzyme, common to Enterobacteriaceae (beta-galactosidase) as techniques to classify raw potable water sources.

TESTS

Bacteriophages are virus-like entities that invade bacterial cells. Guelin in 1948 was the first researcher to properly apprise the potential of bacteriophages as indicators of a faecal pollution. Since Guelin's recognition of the potential of bacteriophage to act as indicator systems, there have been several research reports indicating the potential of bacteriophage/coliphage to act as indicators of microbiological water quality (Besco, 1963; Amin-zade and Poultof, 1964; Kenard and Valentine, 1974; Scarpino, 1975; Wentsel et al, 1982; Grabow et al, 1984; Kennedy et al, 1985). The most detailed and intensive studies on growth and recovery of coliphage can be found in the Atlanta Research Report of 1979 by Scott et al. In an earlier major review of coliphages by Scarpino (1975) he stated "correlations appear to exist in fresh and marine waters between faecal bacterial pathogens, such as Salmonella and Shigella species and faecal indicator such as Escherichia coli and their bacteriophage". Then in 1984, Grabow et al reported "coliphage counts could give a useful estimate of numbers of other microorganisms in sewage polluted waters" and in their studies "evidence is presented, that though counts of coliphages may not always directly correlate with those of enteric viruses, coliphages meet the basic requirements of an indicator for the virological safety of water". Based on the incidence and behaviour of coliphages and enteric viruses in raw water sources, various treatment and disinfection processes, and final supplies, Grabow et al (1984a) have proposed a coliphage limit of 0/100 mL for drinking water, including supplies directly from waste water.

From the studies performed at the Atlanta Research Corporation (1979) and others reported in the recent literature, it would appear that, in the various environmental and drinking waters tested, the coliphage procedure is a reliable indicator of E. coli and coliforms. There is also sufficient evidence to suggest that the coliphage test has many advantages over traditional bacteriological and virological tests in that the procedure is economical, simple to perform and can provide results within six hours of testing.

The correlation/relationship studies between coliphage and bacteria and the inferences drawn from these which are reported above and in the literature may be considered by many to be inappropriate, as there are no direct consistent numerical relationships between coliforms, faecal coliforms, E. coli and the degree of hazard as related to the incidence and infectivity rate of water-borne Salmonella, Shigella, Vibrio cholerae and viruses (Dutka, 1973). Also, there are no consistent and obvious numerical relationships in receiving waters and drinking waters between faecal coliforms, E. coli, Salmonella, Shigella, Vibrio cholerae, viruses and coprostanol, the absolute indicator of faecal contamination (Dutka and El-Shaarawi, 1975).

In all uses of indicator organisms, a concept is used that usually works and is protective (and possibly over-protective) of users of potable and natural waters. The authors of a recent international study (Dutka, et al, 1987) believed that due to increasing stresses on water supplies, rising analytical costs, decreasing budgets, frequency of natural disasters which require immediate responses, e.g. volcanic eruptions, earthquakes, frost upheaval of pipes, etc., the need exists to develop cheaper, simpler and quicker indicator systems which will reflect both bacterial and viral contamination from sewage.

Testing for the inducible bacterial enzyme beta-galactosidase was the second procedure considered at the Singapore meeting. It was believed that there was a potential for the development of a simple test, using this microbial enzyme, to screen for hazardous or indicator bacteria in raw and potable waters. To evaluate this potential, it was proposed (by the Thailand researchers) to add a chemical analog of lactose, one of the substrates for beta-galactosidase, to the water samples, in expectation that any target bacterial cells present would be induced to produce large quantities of this enzyme. It was believed that even if small numbers of Enterobacteriaceae were present, the beta-galactosidase produced would be detectable by a sample colorimetric assay.

This simple two-pronged research proposal escalated as a better awareness of the needs of developing countries in the field of water bacteriology came into focus. It soon became evident that laboratories and trained staff were at a premium, funding for water quality testing was rapidly disappearing due to rising costs and that the majority of rural populations rarely, if ever, had their water supplies tested. Thus, there was an obvious need for simple, inexpensive, reliable, bacteriological tests which could be performed in urban centres as well as in isolated rural communities and under field conditions.

As the number of laboratories involved in this IDRC program increased from the original site in Bangkok to encompass the Southeastern Asian sites of Kuala Lumpur and Singapore and then Lima, Santiago and Sao Paulo in South America, to finally Cairo and Rabat in North Africa, the complexity of the research program also increased.

Initially the faecal coliform Most Probable Number (MPN) 15-tube test using A-1 broth was added to the system to evaluate raw waters as well as a variety of potable water supplies. The A-1 broth test is a simple 24-hour single medium test with an easily recognizable endpoint-gas production, after 22 to 24 hours incubation at 44.5°C. The results obtained by this test were compared to traditional faecal coliform and total coliform procedures used in each country. The A-1 broth test was also used to study the relationship between E. coli, faecal coliform and coliphage.

It was recognized that potable water supplies were not being adequately controlled in many developing countries for a variety of reasons, two of which were the cost factor and the lack of trained personnel. To try to help solve this problem, two extremely simple, inexpensive and reliable procedures were proposed for evaluation in some of the laboratories, the Presence/Absence (P/A) test (Clark, 1968) and the H_2S paper strip test (Manja et al, 1982). Both of these tests are single bottle tests to which potable water is added and then incubated at 35°C for up to five days. Later research results would show that 26°-35°C incubation produced similar results.

The P/A test can be performed using various amounts of media so that a rough quantitative measure may be made. In the routine test 50 mL of media is placed into a screw capped bottle and autoclaved. Then 100 mL of potable water is added, the capped bottle is shaken, then incubated. If the colour of the media changes from red to yellow, a positive result is recorded indicating the potential presence of one or more indicator bacteria (coliforms, E. coli, Pseudomonas aeruginosa, staphylococci or streptococci). Isolation and identification procedures may be carried out on positive samples for confirmation, if desired. In the research studies, positive P/A tests were confirmed by isolate identification.

The H_2S paper strip procedure is equally simple. Filter paper or tissue paper sheets (75-80 cm^2) are impregnated with a simple chemical mixture (1 mL per 75-80 cm^2) which are placed into screw capped bottles and dried under sterile conditions at 50°C. Similar to the P/A test routine quantitative estimates may be made by using different volumes of water and more or fewer cm^2 of impregnated paper strips (not fully tested yet). In the routine tests, 20 mL of potable water are added to the bottle and incubated at 35°C for up to five days. A blackening of the paper strip (usually within 24 hours) indicates the presence of an indicator bacteria, usually Enterobacteriaceae. As with the P/A test, positive results were confirmed by isolate identification.

As new countries were brought on stream, the research projects became more complex. By the time the eighth country became involved in the IDRC coliphage project, samples being collected and tested were: bottled drinking water, with and without gas, potable tap water, drinking water reservoirs and distribution lines, city wells and rural wells, as well as potable water storage vessels ranging from huge standpipes to local earthenware jars with stored rain water. A variety of drinking water source waters were also sampled, usually small lakes or ponds, rivers and ditches. Data from these waters will, at a later date, be combined with sanitary surveys in order to establish a priority ranking of water supply sources.

The four tests being evaluated by this eight country network were controlled by and compared to a variety of procedures used locally on a routine and research basis. The control tests varied from routine membrane filtration procedures using total coliform and faecal coliform media to the use of square gridded membrane filters with special and traditional coliform/faecal coliform media. Various MPN media and procedures (10-tube and 5-tube series) were used to estimate total coliform and faecal coliform densities. Other variables in these studies included testing local E. coli host strains for the coliphage test, evaluating temperature ranges for the P/A, H_2S paper strip and coliphage tests as well as studying the relationship between coliphage, indicator bacteria and human enteric viruses in various waters. A great effort was also expended on isolating and identifying the bacteria from each of the enumeration procedures. This effort was very important in establishing the selectivity and reliability of the various media and procedures being evaluated and compared.

GENERAL OBSERVATIONS

1. The coliphage test was found by the laboratories to be a simple, inexpensive and easy to perform test with the E. coli host ATCC 13706 being the most sensitive in the waters tested.

2. Several laboratories reported that they believed that the coliphage method (with 8 hours incubation) could be used as an indicator of faecal contamination.

3. The majority of the laboratories found that there was a good statistical relationship between coliphage and faecal coliform populations in river and lake waters. However, in some well waters, turbid river waters and rain waters the strong statistical relationship between indicator bacteria (total-faecal coliforms) and coliphage broke down, and this appears to be an area requiring more research to clarify the reasons for this relationship.

4. In one study dealing with a fresh water river, a fresh water lake and marine beaches, it was concluded: (a) that within location faecal coliforms and coliphage are positively correlated; (b) coliphage values can be indicated or predicted by using faecal coliform MPN, faecal streptococci and E. coli data; (c) it would be feasible to propose a water quality guideline of 20 coliphage/100 mL for recreational waters; (d) faecal coliform or coliphage counts in marine waters are not always predictable of the presence of Salmonella and enteroviruses; and (e) in marine waters where pathogens are found, coliphage-pathogen rations are smaller than faecal coliform-pathogen ratios.

5. Laboratories on three continents studying potable water (bottled with and without gas, and treated tap water) found that it was not uncommon to find coliform-free potable water

containing coliphage. Thus, the finding of coliphage in these drinking waters with and without coliform presence, suggests that enteroviruses can also survive the normal treatment and disinfection process accorded these potable water samples (Havelaar, 1986). Another implication of the data from these studies is that coliform-free potable waters are not necessarily pathogen-free potable waters. Therefore we suggest, based on these studies, that coliphage tests be included as part of any potable water testing schemes.

6. The general conclusion from these studies is that the coliphage test has an advantage over traditional microbiological tests, in that it can be read after six hours incubation. The procedure is very economical and easy to perform and its sensitivity can easily be increased by testing more 5 mL aliquots or by using a coliphage MPN technique or even by using a large petri dish and testing 100 mL of sample.

7. In all laboratories where the A-1 broth test for faecal coliforms was evaluated it was usually found to be the most sensitive technique and produced higher faecal coliform counts than any of the other media and procedures that were compared. In the rare instance when the A-1 test was found to only produce equivalent results to traditional local faecal coliform population estimation techniques, it was found that the A-1 test had a cost and time advantage over the other faecal coliform estimation tests.

8. Isolates collected and identified from positive A-1 broth MPN tubes were found to be _E. coli_, 80-100% of the time. Thus, the A-1 broth procedure can provide laboratories with an approximation of the _E. coli_ concentration in raw water supplies and recreational waters.

9. It was concluded by several countries that the A-1 broth technique should be considered as the preferred bacteriological test for the examination of raw water supplies and recreational waters and the A-1 test, combined with the coliphage test, would make an excellent screening program for health hazards in raw water supplies (Castillo, 1988).

10. The Presence/Absence (P/A) test was found by all laboratories using the procedure, to be the most sensitive and cost effective means of testing potable water supplies (bottled and tap) for bacteriological contamination. In many instances, potable waters which were negative by traditional MF and MPN coliform estimation techniques, would be found positive by the P/A test.

11. The P/A test was found to be a very portable test in that bottles of media could be transported anywhere without refrigeration and the sample could be collected and tested by untrained personnel. This factor, coupled with its low cost and minimal storage requirements, makes it the ideal test for potable water safety anywhere in the world. The P/A test can be made partially quantitative by preparing smaller volumes of media, e.g. 5 mL, 12.5 mL and 25 mL and thus 10 mL, 25 mL and 50 mL of water sample could be tested.

12. In summation, from this three continent study the superiority of the sensitivity of the P/A test for monitoring potable water samples can easily be seen. This test is relatively inexpensive, simple to perform, and is recommended without reservation for all routine water quality analyses. We believe this procedure can enhance water quality testing procedures, especially when cost is a factor (Castillo, 1988).

13. The H_2S paper strip technique for testing potable water supplies was found, by the majority of laboratories, to be equivalent to or slightly less sensitive than their traditional/routine potable water testing procedure for coliforms.

14. Several of the laboratories indicated that the paper strip procedure would be ideal for testing rural and isolated drinking water supplies as well as local urban potable waters. The medium (impregnated paper strip) has an unlimited shelf life and the test procedure requires no training for collecting the sample and interpreting the results.

15. Since the H_2S paper strip technique is based on the testing of only 20 mL of water samples while the other MF, MPN and P/A tests use either 55.5 mL or 100 mL of sample, there is a possibility that with research into media concentrations versus sample volume, the H_2S paper strip could be used to test 100 mL of sample, thus perhaps making it compatible to the P/A test in sensitivity.

16. In summation, the H_2S paper strip test is probably the best and simplest method to test remote water supplies, as well as for use in city and town laboratories. It is believed that the P/A and H_2S paper strip techniques combined with the coliphage test, would provide an excellent assessment of the safety of potable waters from bacterial and virus contamination.

ADDENDUM

In 1988, these simple, inexpensive microbiological water quality assessing procedures described above were used by a remote Canadian Indian Band to test and monitor their recreational and drinking water supplies. This pilot project has proven to be a major success story with the Band members realizing that they now have the ability to control the quality of their own water supplies.

It is expected in 1989 that this pilot project will be expanded, at the request of other Indian Bands. Staff from the federal Departments of Environment and National Health and Welfare are monitoring and assisting this technology transfer.

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DID THE IDRC WATER QUALITY CONTROL PROJECT ACHIEVE IT'S OBJECTIVES?

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ABSTRACT

Some comments are made concerning the statistical interpretation of the collected data from the International Development Research Centre (IDRC) Global Water Quality Control Project. Special attention is directed to: the type of statistical analysis required, the issues involved in combining data from a variety of sources (countries, types of water, etc.), and the determination of the range of applicability of each bacteriological test.

INTRODUCTION

In September 1988, a meeting was held in Banff, Alberta, Canada, to evaluate the results achieved from the Global IDRC Water Quality Control Project and to determine directions for both research and applications. The general objectives for the project were to identify a rapid, inexpensive and simple test for estimating the bacteriological qualities of potable water sources and to develop an objective criteria using the generated data along with information obtained from sanitary surveys of raw and potable water to classify the water sources into a set of homogeneous categories of different levels of risk to the user's health. The achievements of the objectives depend largely on: (a) data quality; (b) methods used for analyzing the data; and, (c) the range of applicability of the results. The data presented in the Banff meeting and the papers given in this volume show, in many instances, discrepancies among the results from the different countries and where there are agreements, the strength of evidence is variable among the countries. For example, the Brazilian study indicates no significant correlation between faecal coliform and coliphage for untreated well water, while in other countries the correlation was statistically significant. This raises the questions: (1) what are the causes of these differences?; and, (2) is it possible to combine results from different countries or different water sources? To answer these questions adequately factors (a), (b) and (c) need to be discussed.

DATA QUALITY

Planning and executing the data collection are the two limiting factors in determining the data quality. The planning is dependent on bacteriological, logistical and statistical factors. The choice of the target organisms (coliform, coliphage) for monitoring the bacteriological tests to be used in the study, the sample volume to be analyzed, etc., are among the bacteriological factors. The level of technical and nontechnical efforts, as well as the costs involved in performing the study are examples of logistical factors. The determination of the target population for which the results can be applied and of the level of precision required in the study represent the statistical consideration. One major difficulty with the study was the absence of a comparative quality assurance program among the participants. So when a discrepancy occurs, it is then difficult to assign a specific cause for it, i.e. are the differences due to differences in laboratories or in the physical and chemical characteristics of the water sources?

In each study, the investigators used samples from a variety of water sources in their countries. As a result, assuming that the bacteriological analyses were well executed, their conclusions should be applicable to the majority of their water sources. Furthermore, bacteriological tests were performed in triplicate and this allows the calculation of a measure of uncertainty or reproducibility of the results.

Difficulties encountered with the data sets include the presence of both right and left censoring. The causes for these can be attributed to the nature of bacteriological tests and the volume of water sample used. Also, there were many missing data points. The effects of these are: (1) to complicate the statistical analyses; and, (2) to reduce the precision of the results.

METHODS OF DATA ANALYSIS

In general, the methods of data analysis have to be tailored towards: (1) achieving the stated objectives of the investigation; (2) extracting all available information from the data; (3) identifying areas where more information are required; and, (4) designing future data collection. Inspection of the methods of analyses and the level of data interpretation indicate more work needs to be done in this area. Prior to presenting a strategy for data analysis, it is appropriate to list at the outset the objectives of the data analysis. These are to determine:

1. the range of applicability of each test;
2. the degree of precision that can be attached to each test;
3. an estimate for the uncertainty associated with the use of a specific test for characterizing water quality;
4. the degree of overlap and the level of association among different tests; and,
5. the number of tests and the number of determinations required for each test that can adequately describe the variability of the different water sources.

Achieving these objectives requires both the applications of exploratory and confirmatory types of statistical methods. Graphical methods are the basic tools at the exploratory stage, while formal statistical estimation and hypotheses testing methods are used at the confirmatory stage. A brief description of these techniques and their goals is given in the following.

Graphical Methods

Graphs are very useful in showing the distributional characteristics of the data, the associations among variables and the identification of unusual observations. It is instructive to distinguish between graphs which are designed to show the distributional nature of the data and graphs which are appropriate for detecting the existence and nature of association in the data.

1. Graphs for Determining the Data Distribution.

Graphs of this type include the histogram, the cumulative probability distribution function, the box plot, and different types of probability plots, e.g. p-p plot and Q-Q plot (Cleveland, 1985). These types of graphs are useful also for determining unusual (outliers) observations.

2. Graphs for Displaying Association Among Multivariate Data. These include the scatter diagram matrix, the box plots, Anderson's glyphs, Kleiner-Hartigan trees, Chernoff's faces and Andrew's plots. Some of these techniques have been used very effectively during this project.

3. Estimation of the Density of an Indicator.

There are two types of bacteriological tests used during the project. One type consists of those tests that are designed to measure the density of a bacterial indicator (e.g. faecal coliforms) while the other is concerned with the presence or absence of an indicator or a group of indicators. Issues of concern include: (a) the estimation of the density of the indicator in the water source and its standard error or confidence limits; (b) the comparison of the bacterial density or the proportion of positive samples to a prespecified standard or a regulatory criterion; (c) the comparison of densities of an indicator as estimated by different bacteriological tests; (d) the measurements of the association among different bacteriological tests; (e) the assessment of the influences of the physical, biological and chemical characteristics of the water source and the adjustments of the comparison between densities so that the effects of these influences in the comparison can be eliminated or reduced; and, (f) the determination of a subset of tests that can adequately describe most of the variabilities in the water sources of each country. Below is given a brief description for each of these issues.

(a) **Estimation of the density of an indicator:** There are a number of models that have been found by many investigators (El-Shaarawi and Pipes, 1983; Maul et al, 1985) to adequately describe the variations of the bacterial densities in raw and drinking waters. These include the Poisson, negative binomial, Poisson plus added zeros, and the log normal distributions. Optimal estimates are dependent on the distribution used to represent the data. Hence, it is important to use the data to choose an adequate model not only for obtaining optimal estimates, but also for deriving correct confidence limits. No serious attempts were made to determine what type of distribution is adequate to describe the data.

(b) **Comparison of indicator density or proportion to a specific value:** This can be accomplished within the frame of a specified model or it can be done without assuming a particular model. The former is known as a parametric testing while the other is known as nonparametric testing. Matched paried t-test, analysis of variance are examples of parametric methods, while the sign test, McNemar test, Wilcoxen signed rank test are examples of nonparametric tests. Some uses were made during the project of both parametric and nonparametric approaches. However, the methods used may not be the most adequate ones.

(c) **Comparison of bacteriological tests for measuring the same indicator:** The most commonly used method for comparing the techniques was linear regression and correlation. The validity of such a method depends on the acceptance of the linearity and on how well the variability of the data can be represented by the normal distribution. These issues were totally ignored in the data analysis.

(d) **Association among different tests not measuring the same indicator:** Parametric and nonparametric techniques were used to study the association. Difficulties in using these techniques are the same as given in (c) above.

(e) **The effects of the physical, chemical and biological factors on the results of the bacteriological tests:** Although several factors were thought to cause variation in bacteriological densities estimated by the different tests, no attempt was made to quantitatively estimate or eliminate their effects on comparing the performance of different techniques.

(f) **Determination of a subset of tests that can be used to describe adequately the quality of water:** This issue requires the utilization of many advanced multivariate statistical techniques for measuring the information loss that can result from reducing the number of bacteriological tests used for the examination of water samples. The results of such reductions will provide an important simplification to the issue of the classification of the water sources. This type of analysis was not performed in any of the studies.

RANGE OF APPLICABILITY

It is hoped that the data generated from this project will identify a single test or a small subset of tests which are simple, economic and rapid to be used for the universal monitoring of potable water sources and this is an important reason for conducting these comparative studies in seven countries from three continents. There are two approaches for achieving this generalization. The first approach assumes that the data were generated by some random mechanism at the design stage, while a model based inferences is required for the use of second approach. It is clear that the design used in the studies in all countries does not permit us to use the first approach since the water samples were not chosen according to a random mechanism. Hence, only a model based approach is appropriate in these studies. This can be done by adequately analyzing the data from each country and then integrating the data analysis across countries.

CONCLUSION

The project has resulted in generating a unique data set on traditional and nontraditional bacteriological tests which is likely to be very valuable scientifically and for use in developing economically viable approaches to managing the quality of water sources in developed and underdeveloped countries. The project has resulted in generating a unique data set on traditional and nontraditional bacteriological tests which is likely to be very valuable scientifically and for use in developing economically viable approaches to managing the quality of water sources in developed and underdeveloped countries.

The project has resulted in exchanges of scientific and field information among researchers from the countries involved in the project. Also, several research articles and reports have been published describing certain aspects of the project and/or listing the raw data along with the methodological bacteriological work. In some countries it was possible to identify potential appropriate tests for monitoring their water.

More work is needed for interpreting the generated data from each country and on integrating the data from the entire project. This will lead to the identification of future research needs, the scope of each test and reliability associated with the application.

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EVALUATION OF THE COLIPHAGE DETECTION METHOD FOR SOUTHEAST ASIAN WATER QUALITY CONTROL

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ABSTRACT

The drinking water supplies in rural areas in Southeast Asia are often drawn from small water catchments such as wells, holding tanks, ponds and from rivers. In order to maintain water quality standards in rural communities, it is necessary to have a simple, rapid, cheap and reliable technique for monitoring the bacteriological quality of such drinking water.

The coliphage detection method was evaluated concomitantly with conventional water quality tests for faecal coliforms, viz. most probable number multiple-tube fermentation broths (LST, BGB, EC and A-1) and membrane filtration (MFC) method. Over 1,000 water samples collected from the three collaborating countries, Malaysia, Singapore and Thailand, were analyzed. There is significant correlation between coliphage numbers and faecal coliform numbers in river water and in water from large ponds, in that coliphages are found as frequently as faecal coliforms. However, statistical analyses have also shown that correlation between coliphages and coliforms was significantly affected by turbidity. The detection of coliphages in faecal coliform-free tap water samples (22%) in Thailand suggests that coliphages are relatively more resistant to the chlorination treatment of municipal drinking water. This, in turn, suggests that faecal coliform-free water is not necessarily pathogen-free and that coliphages might be more useful indicators for water quality.

Attempts made to improve the coliphage detection method indicated that the coliphage test gave better results when incubated at ambient temperatures and that local isolates are potentially useful. Based on comparison of all the water quality tests for cost-effectiveness, reliability and simplicity, the coliphage test is considered to be the best method for application in rural laboratories in Southeast Asia.

INTRODUCTION

A three-country collaborative project involving Malaysia, Thailand and Singapore was initiated in 1984 to develop a simple, rapid and reliable bacteriological test for evaluating drinking water quality in rural areas.

In Singapore, water supplies come from local surface water catchment and from Johore, the nearest State of our neighbouring country, Malaysia. The drinking water supplied is regularly treated and examined by conventional methods. Drinking water from reservoirs are treated and examined by conventional methods. Drinking water from reservoirs are treated by conventional methods involving flocculation with aluminium sulphate or lime, primary settling, sand-filtration and chlorination. Ozone is used in addition to chlorination in some treatment plants. Activated carbon is also used to remove objectionable taste and odour. Fluoridation has also been practiced as a prevention against dental decay in children (Anon, 1985). Daily chemical and standard bacteriological tests (e.g. MPN and MFC) are conducted to ensure water quality standards stipulated in the International Standard for Drinking Water set by the World Health Organization (WHO) are met.

In Malaysia and Thailand, most of the water currently used comes from surface water supplies and rainwater catchment. Although municipal water supplies in Malaysia and Thailand undergo similar treatment regimes and biochemical testing, the water supplies in many rural areas are untreated, for example, well water, pond water and gravity-feed water supplies from rivers and ponds. Thus, it is the concern of public health agencies to ensure the safety of water used in rural areas. The contamination of water sources by man is one of the major causes of water-borne diseases such as typhoid fever, cholera, dysentery and infective viral hepatitis. In order to reduce the proliferation of such diseases, it is crucial to maintain and monitor the sanitary quality of drinking water supplies. One of the basic necessities is to have a rapid, simple and reliable bacteriological test to assure the quality of such water supplies. A classification scheme can then be established to categorize various water sources for their intended use according to the levels of contamination.

Although the causative agents (bacterial, parasitic or viral) for water-borne diseases are known, it would be impossible to monitor their presence routinely, either due to the high cost or difficulties in carrying out specific tests or the length of time required for the detection of most of the potential pathogens. Thus, indirect methods based on the presence of indicator organisms which infer the likely presence of pathogens are used routinely, worldwide.

Conventionally, the detection of indicators such as total and faecal coliforms has been used to determine the bacteriological quality of water. However, the methods used have been developed for use in temperate countries having different environmental and socio-economical factors compared to tropical countries. In addition, the high cost and skill-intensive nature of the conventional coliform tests renders them unsuitable for use in many developing countries lacking capital resources and expertise.

In many developed countries, water sources and supplies are centralized, thus treatment and surveillance can be effectively carried out before supplying to end-users. In many Southeast Asian countries, however, water supplies are often decentralized, i.e. rural communities are dependent on their own limited water supplies such as wells, holding tanks, ponds and nearby rivers. Thus, the monitoring of water quality cannot be easily carried out based on conventional coliform tests. In view of such problems, the coliphage detection method was evaluated as a means of monitoring water quality in Southeast Asian waters. Coliphages are known to infect and replicate in coliform bacteria. The presence of coliphages in a water source would also indicate that coliforms are also present. Coliphages and coliforms have been found to correlate reasonably well in natural waters (Isbister et al, 1983). In order to set realistic standards based on coliphage presence, it was necessary to compare and relate the coliphage detection method with several conventional methods for coliform detection.

To this end, at least 1,000 samples have been collected for over twenty months from several water sources in the three countries involved so that the natural trends of the coliphages and faecal coliforms in Southeast Asian waters can be studied and the results used to prioritize drinking water sources based on bacteriological water quality.

Laboratory Methods Used in the Study

All methodologies were standardized for the three countries so that comparisons could be made.

The variety of water sources examined were river water, shallow wells, deep wells, pond water, hand-pumped water, rain water and tap water. The enumeration of total coliforms and faecal coliforms was according to the APHA Standard Methods (1985) for the multiple-tube fermentation enrichment method (MPN) and the membrane filtration method (MFC); and according to the Methods for Microbiological Analysis of Waters, Wastewaters and Sediments (Dutka, 1978) for the A-1 MPN broth method.

Coliphages were enumerated based on the ARCAT method as detailed in APHA Section 919C (1985). Enumeration of coliphage is based on the number of plaque forming units or zones of clearing formed within the lawn of bacterial host cells (*E. coli*) plated on an agar medium. Each plaque is the result of multiple events of bacterial host cell lysis following the infection of a virulent coliphage into a susceptible bacterial cell. The test is carried out simply by mixing in a tube, 5 mL of water sample (prewarmed to 45°C) with 5.5 mL of molten modified trypticase soy broth (MTSA) supplemented with strontium nitrate and ammonium nitrate. Both strontium nitrate and ammonium nitrate have been reported to increase the efficiency of plaque formation. In addition, 80 mL of a 1%

solution of 2,3,5 triphenyl tetrazolium chloride (TPTZ) and 1 mL of an overnight culture of E. coli C host strain (ATCC 13706) were added to the molten agar and the contents mixed well before pouring into an empty petri dish. For each water sample, four tubes, equivalent to testing 20 mL of water sample were prepared and the total number of plaques obtained from the four plates was multiplied by a factor of 5 to give the number of plaque forming units (pfu) per 100 mL of water sample. The use of TPTZ enhances the contrast of the plaque against a pink-coloured bacterial lawn.

RESULTS AND DISCUSSION

Comparative Evaluation Between Coliphage Detection Method and Faecal Coliform Enumeration Methods

Six water sources were evaluated for their bacteriological content. The type of water sources examined was typical of the drinking water used in Malaysia and Thailand, particularly for the rural areas. Untreated pond water samples were included in the Singapore study in order to compare and correlate the tests with water sources examined in Malaysia and Thailand (**Table I**).

Good correlation was observed between coliphage content and faecal coliform content in pond water samples examined both in Malaysia and in Singapore. The data obtained from Malaysian river water samples were plotted (**Figures 1A** and **1B**), and statistical analyses gave the following regression equations based on logarithmic transformation:

(1) log (total coliform MPN) = 1.130 log (coliphage no.) + 1.521

(2) log (EC faecal coliform MPN) = 1.107 log (coliphage no.) + 0.956

(3) log (A-1 faecal coliform MPN) = 1.154 log (coliphage no.) + 0.344

(4) log (MFC faecal coliform count) = 1.133 log (coliphage no.) + 0.816.

Similar experiments were also carried out on pond water samples in Singapore to investigate the effects of six, eight and twenty-four-hour incubation periods on coliphage counts. In the study, the coliphage counts were measured against three conventional bacteriological water tests, viz. A-1 broth 5-tube MPN procedure, LST-BGB-EC 5-tube MPN procedure and MFC membrane filtration test. The results of this study are shown as a correlation matrix using the logarithmic form of the data obtained (**Table II**). The correlation matrix relates each parameter with one of the other parameters sequentially. The best correlation between bacterial counts and coliphage counts appears to be between A-1 broth and eight and twenty-four-hour coliphage counts. It is also clear that the coliphage enumeration correlates well with all the MPN enumeration and the MFC faecal coliform counts. These data are supportive of the view that the coliphage test can be used with confidence to gain similar health hazard information as provided by the MFC and MPN faecal coliform tests.

Of the three methods used for faecal coliform detection, viz. MPN-EC faecal coliform, MFC-faecal coliform and A-1 broth faecal coliform tests, the A-1 broth method was found to be the most representative of faecal coliforms since 80-90% of the positive tests were E. coli, with the remainder being Citrobacter freundii and Klebsiella pneumoniae. In comparison, 70% of the faecal coliforms enumerated by the membrane filtration method were E. coli, and 30% were mainly Klebsiella spp. and Citrobacter sp.

However, similar analyses carried out on data obtained for testing coliphage and coliform content in water samples examined in Thailand, gave poor correlation between the variables studied, as shown in **Table III**. In relative terms the standard error of coefficient increased in the order: shallow pond water to deep pond water to rain water.

In the case of tap water in Thailand which is normally chlorinated, faecal coliforms were detected at lower concentrations compared to those of other sources. However, the detection of coliphages in tap water that were coliform-free (22% of samples) indicates that the chlorination procedure merely eliminated coliforms but not coliphages. Due to the differences between bacterial and coliphage resistance to chlorination, the results of the above study suggest that other water-borne viruses may also be unaffected by the chlorination used. Since coliform-free water may not be necessarily pathogen-free, it would be advisable, based on these studies, to augment bacteriological drinking water with coliphage tests.

Effect of Physio-chemical Factors on the Coliphage Test

The data have shown that a correlation between coliphages and coliforms exists in water samples analyzed in Southeast Asia, particularly in river water and large ponds. However, weaker correlations were also observed in other water sources, for example, rain water and shallow ponds. In order to test the effect of environmental factors on these correlations, a large number of samples collected from the Langat River in Malaysia were analyzed by multiple regression analysis of the various parameters, for example, pH, dissolved oxygen, turbidity and coliphages. It is apparent from **Table IV** that the turbidity of samples had the most significant effect on coliphage enumeration. Dissolved oxygen and pH had a less significant effect on the coliphage test. These observations could possibly account for the poor correlations between bacterial counts and coliphage counts in water samples collected from rural gravity feed systems and hand-pumped water in Malaysia, and also the well water, rain water and pond water in Thailand.

Micro-organisms including viruses have been found to associate with solid particles and clay-like materials leading to their accumulation and settling to the bottom sediments of an aqueous environment (Bitton, 1980). This suggests that in certain cases, coliphages may be present, but are concentrated at the lower depths of the water body. Consequently, lack of or low correlation between coliforms and coliphages may be seen within a sample taken from settled water sources without good vertical mixing.

Improvements to the Coliphage Test to Suit Local Conditions

In attempting to improve the coliphage test, three factors were considered. Firstly, to test the effect of incorporation of sodium dodecyl sulphate (SDS); secondly, incubation of the test plates at ambient temperatures and thirdly, using local E. coli isolates as the bacterial host for plaque formation.

Sodium dodecyl sulphate (SDS) was incorporated in the coliphage water test on the assumption that SDS may affect the cell membrane and so enhance the susceptibility of the host cell. Protein subunits, for example, receptors for coliphage binding, which may be present on the surface membrane could be altered chemically or structurally, resulting in a change in the specificity and affinity of the protein subunits. Since coliphages are likely to utilize these protein subunits for their attachment onto the host cell, treatment with SDS could influence the infectivity. The experimental results (**Figure 2**) showed that increasing SDS concentrations did not improve the recovery of the coliphage stocks used (phage 0X174 and two local isolates, OMP and OIIIB). Conversely, there was a significant decrease in coliphage recovery with increasing SDS concentration from 0.005% up to 0.05%.

In order to adapt the ARCAT coliphage test for field conditions, we investigated the possibility of performing the test at ambient temperatures instead of at 35°C constant incubation temperature (**Table V**). The results indicated that the use of a constant temperature incubator was not necessary and that quite often higher plaque counts were obtained in tests carried out at ambient temperatures. The tests carried out in Malaysia and Singapore gave similar results. Thus, incubation under field conditions (26°C+) should produce reliable coliphage density estimates and would be economical since the use of a constant temperature incubator is made redundant.

E. coli C has been found to be suitable as the host for coliphage formation in the ARCAT procedure by nature of its broad host range and restrictionless properties. Highly significant correlation between coliforms and coliphage numbers have been reported for studies carried out in the United States (Wentsel et al, 1982; Isbister et al, 1983). However, based on the assumption that local strains of E. coli might be more adapted for the proliferation of coliphages present in Southeast Asian water, fourteen local strains of E. coli were isolated and tested as host organisms for coliphage formation. In comparison to E. coli C as the host strain, the coliphages enumerated and the correlation between coliphages and faecal coliforms did not differ significantly between the different host strains tested. However, the E. coli S4 isolate was found to give larger plaque sizes than those obtained with E. coli C. This would make recognition of plaques, and hence its detection, easier. However, this strain has not yet been tested under field conditions.

CONCLUSIONS AND RECOMMENDATIONS

A technical and cost comparison of the water quality tests used in the Southeast Asian project is presented in **Table VI**. At a glance, it is evident that the coliphage test is competitively useful and fulfils the need for a simple, rapid, reliable and inexpensive method for monitoring water quality.

Both the A-1 broth MPN test and the coliphage test are relatively inexpensive in terms of consumables required. However, the coliphage test is the simplest to perform and does not require special equipment.

All the MPN and MFC tests provide information on the presence of faecal coliforms. However, the detection of the presence of coliphages indicates prior infection of faecal coliforms. The coliphage test is relatively simple to perform, involving the suspension of a total of 20 mL of water sample in E. coli seeded agar prior to incubation at ambient temperatures for six to eight hours. Hence it can be easily adapted for rural laboratories or field work. The coliform test methods require basic aseptic techniques and the suitability of each method for different types of water need to be a priori determined, for example, the MFC method would be unsuitable for turbid water found in shallow ponds.

It is well known that the survival of microbial species in water is greatly influenced by their physicochemical environment. Thus, it is not surprising to find that the differences in water type and water sources found in Southeast Asia are related to the frequency of detection of coliphages and faecal coliforms. Studies on the influence of physicochemical parameters on the coliphage detection test may be beneficial in improving the sensitivity of the test when applied in the many different water found in

Southeast Asia. On the other hand, since the coliphage test is dependent on a stable E. coli host, a comparative study on the survival, efficacy and plaque size produced using different host organisms (including locally isolated species) under field conditions would also be useful. Lastly, the relationships, whether direct or indirect, between water quality indicators such as coliphages and coliforms, and the toxicity assessment of water sources are likely to yield valuable information towards the classification of water sources in rural areas.

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TABLE I

TYPES OF WATER SOURCES EXAMINED FOR BACTERIOLOGICAL WATER QUALITY

COUNTRY	WATER SOURCES EXAMINED
Thailand	Ponds (shallow and deep), wells, tap, and rain water tanks.
Singapore	Ponds, wells, raw water reservoir and tap.
Malaysia	Ponds, river, hand-pumped water.

TABLE II

CORRELATION MATRIX OF POND WATER TRANSFORMED TO LOGARITHMS

	Coliphage 6*	Coliphage 8*	Coliphage 24*	EC-Faecals	A-1 Faecals	MFC-Faecals
Coliphage-6	1.00000					
Coliphage-8	0.94192	1.00000				
Coliphage-24	0.91736	0.99712	1.00000			
EC**-faecals	0.54555	0.64599	0.65586	1.00000		
A-1**-faecals	0.54391	0.66600	0.68121	0.95788	1.00000	
MFC**-faecals	0.43233	0.57723	0.60060	0.84341	0.84953	1.00000

Note: *These numbers correspond to the detection of coliphages after 6, 8 and 24 hours of incubation.

**These are faecal coliform enumeration methods as described in the Materials and Methods section.

TABLE III

CORRELATION OF LOGARITHM OF FAECAL COLIFORMS AND LOGARITHM OF COLIPHAGES

Source	Incubation [hours]	Variables	Standard Error of Coefficients
shallow pond water	6	EC-faecals : coliphage	0.2
"	6	A-1-faecals : coliphage	0.23
"	8	EC-faecals : coliphage	0.19
"	8	A-1-faecals : coliphage	0.22
rain water	6	EC-faecals : coliphage	0.5
"	6	A-1-faecals : coliphage	0.49
"	8	EC-faecals : coliphage	0.37
"	8	A-1-faecals : coliphage	0.4
deep pond water	6	EC-faecals : coliphage	0.32
"	6	A-1-faecals : coliphage	0.36
"	8	EC-faecals : coliphage	0.32
"	8	A-1-faecals : coliphage	0.35

TABLE IV

MULTIPLE REGRESSION ANALYSIS OF PHYSICOCHEMICAL PARAMETERS WITH COLIPHAGE ENUMERATED

		Coliphage	Enumerated After	
	4 hours	6 hours	8 hours	24 hours
pH	P = 0.8181	P = 0.0220	P = 0.0040	P = 0.0083
dissolved oxygen	P = 0.0268	P = 0.0087	P = 0.0309	P = 0.1427
turbidity	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0001

TABLE V

RESULTS COMPARING COLIPHAGES AT INCUBATION TEMPERATURE AND ROOM TEMPERATURE

	E. COLI C 35°C			*E. COLI* C ROOM TEMP			FAECAL COLIFORM		
COLIPHAGES	6	8	24	6	8	24	E.C	A-1	MFC
	50	50	50	25	50	50	480	480	780
	50	50	50	75	75	75	92	66	320
	5	5	5	50	100	100	48	48	450
	25	25	50	25	25	100	260	340	350
	25	50	50	25	25	50	66	66	250
	425	500	500	25	75	75	98	46	50
	10	25	25	100	150	150	46	66	270

	S4 35°C			S4 ROOM TEMP			FAECAL COLIFORM		
COLIPHAGES	6	8	24	6	8	24	E.C	A-1	MFC
	5	5	5	15	15	15	480	480	780
	50	100	100	25	50	50	92	66	320
	25	25	25	100	100	100	48	48	450
	50	100	100	150	200	200	260	340	350
	25	50	75	50	50	75	66	66	250
	25	25	25	25	50	75	98	46	50
	10	20	20	50	50	100	46	66	270
	50	75	100	150	200	200	1750	1400	1625
	275	275	275	150	200	225	8000	1400	2500
	50	50	50	50	50	50	1600	1100	1250
	50	50	50	50	50	50	95	95	285
	5	5	5	50	50	50	133	133	380
	25	40	40	25	25	25	627	323	532
	5	5	5	25	25	25	38	38	0

TABLE VI

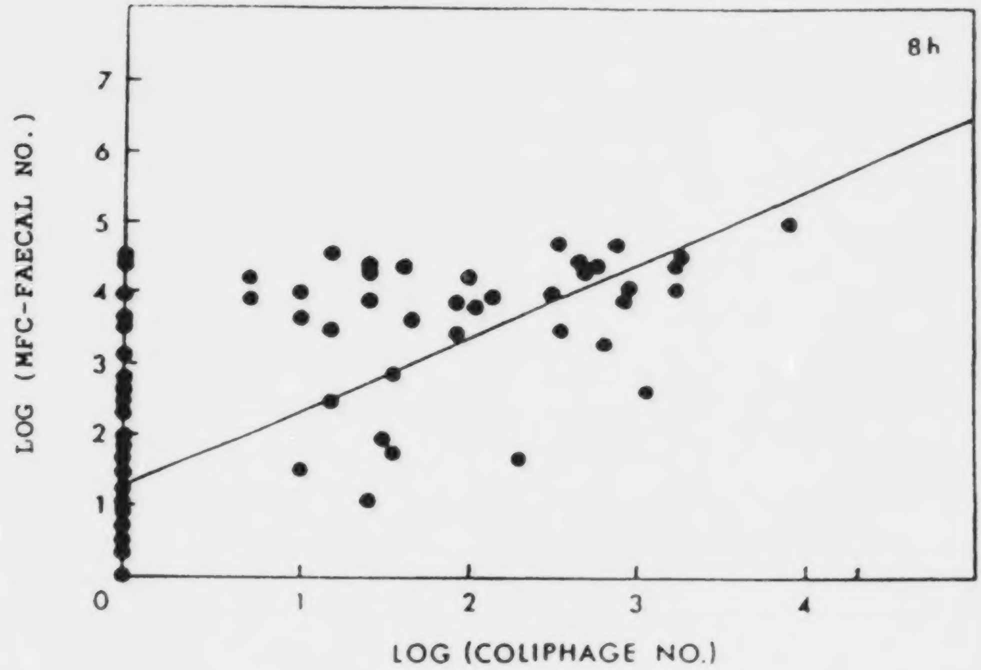
COST AND TECHNIQUE EVALUATION OF DIFFERENT WATER QUALITY TESTS

Method	Time Required	Equipment Cost (relative)	Consumables Cost/Test*	Comments
MPN	24-72 hr	Moderate	S$ 0.54	tedious
A-1 broth	24 hr	Moderate	S$ 0.12	tedious but highly selective for faecal _E_. _coli_
MFC	24 hr	High	S$ 0.73	filtration affected by turbid samples, can be time-consuming
ARCAT coliphage	6-8 hr	Minimal	S$ 0.13	simple, rapid procedure but can be affected by turbidity

* Cost indicated here is for consumables (media) only and excludes glasswares, equipment cost and maintenance.

FIGURE 1

SCATTER PLOTS RELATING LOGARITHMS OF MFC-FAECALS AND COLIPHAGE NUMBERS



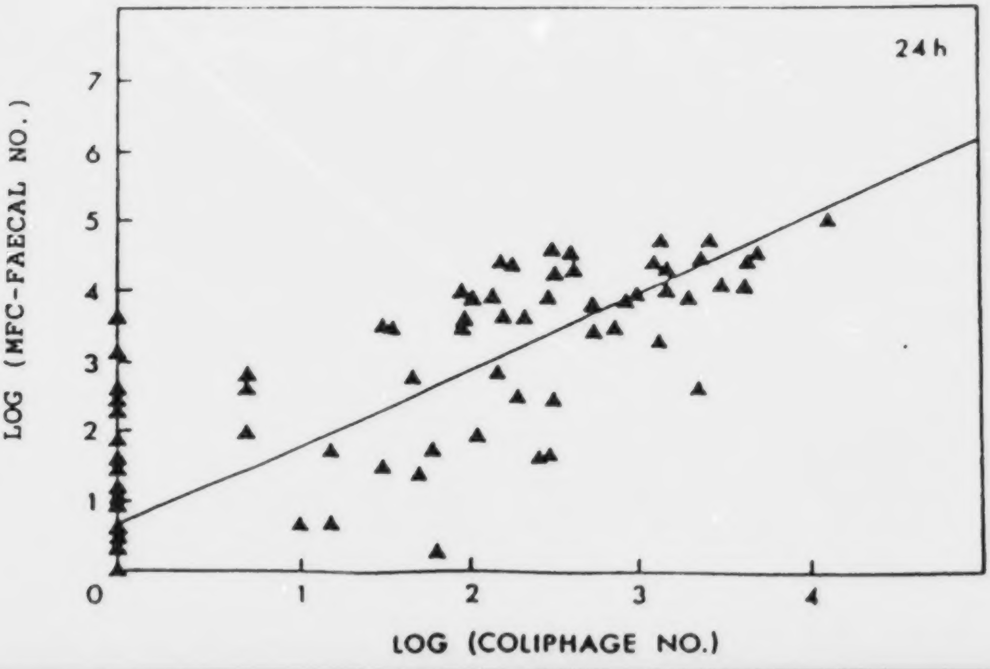
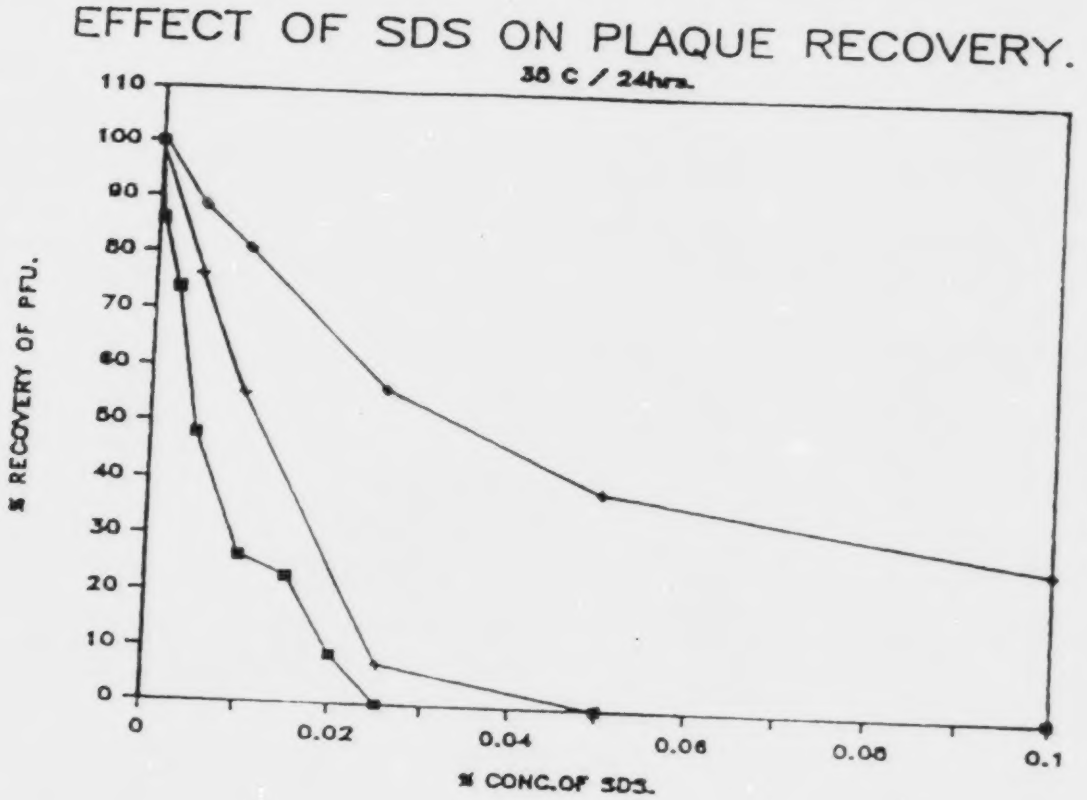


FIGURE 2

THE EFFECT OF SODIUM DODECYL SULPHATE ON THE RECOVERY OF PLAQUES OF THREE COLIPHAGE STOCKS



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EVALUATION OF THE COLIPHAGE PROCEDURE AND PRESENCE/ABSENCE TEST AS SIMPLE RAPID ECONOMICAL METHODS FOR SCREENING POTABLE WATER SOURCES AND POTABLE WATER SUPPLIES IN CHILE

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ABSTRACT

A study was done in the Chilean Central Zone to evaluate the A-1 faecal coliform test and coliphage test for classifying potable water sources, and the Presence-Absence (P/A) and H_2S tests for screening potable water. Good correlations between faecal coliforms, tested by A-1 test and conventional MPN and membrane filtration methods where found in 126 triplicate samples from different raw waters. The A-1 test showed high sensitivity and specificity, and based on its ease of performance, inexpensiveness and rapidity of results, this procedure could be used instead of conventional tests for classifying Chilean potable water sources. Irregular recovery of coliphages from these waters was noted. Coliphage densities tended to vary seasonally and in waters with high turbidity and hardness. P/A and H_2S tests were very effective in screening coliforms and other indicators. Although coliphage were present in raw water, they were never found in drinking water where free residual chlorine was present. The combination of P/A and coliphage tests as rapid, inexpensive and simple tools for assessing the safety of potable water in Chile was demonstrated. The H_2S test also proved to be a good method for assessing the water quality of potable water in rural areas in Chile.

INTRODUCTION

Chile is a long, narrow country on the western coast of South America from 17° 30'S to 55° 59'S, which has a great variety of climates because of its complex geography. Its population is about 11 million, of which approximately 20% live in rural areas.

In order to attain the goals of the United Nations Program for Drinking Water and Sanitation Decade, the Government has been trying to supply the population with safe water in adequate quantities. Approximately 95% of the urban population is supplied with potable water, and efforts are being made to supply the rural population with safe potable water.

A National Rural Drinking Water Program is being developed by the national agency, National Service of Sanitary Works (SENDOS), through which, by December 1987, approximately 19% of the country's rural population had been supplied with potable water supplies. The goal of the project is to supply all of the rural concentrated population (about 600,000 people) by 1990.

Although there has been good progress towards the goals for providing water to the rural areas, there has been, also, a recognition of the inability to appropriately monitor and control the safety of these water supplies.

Water-borne diseases are endemic in Chile. High morbidity rates from infectious hepatitis (HAV) and typhoid fever are recorded yearly; 102/100,000 people with HAV, and 54/100,000 people with typhoid fever cases, were reported during 1987 (Ministerio de Salud, Bol. En Fermedades In Fecciosas, 1988).

Viral, amoebic and bacterial diarrhoea are also common amongst the children of the country, but official morbidity data are not available.

In Chile, potable water is regulated by the National Standard Institute (INN) through Standard NCh 409 of 84, and the bacteriological standards are based on the coliform test (INN, 1984).

Water quality control is performed by SENDOS which is the main water supplier in Chile. In addition, the Ministry of Health is in charge of ensuring that SENDOS and private water agencies perform National Standard Regulations tests.

For controlling the water quality of services, SENDOS has 12 regional laboratories throughout the country for testing and maintaining water quality.

The Ministry of Health ensures that in Chile, urban drinking water surveillance is being done according to National Standard Regulations. Data indicate that over 95% of the 409 urban supplies are being tested (Ministerio de Salud, Diagnostico Sit. Ambiental, Chile, 1987).

Quite different is the situation on potable water quality control in SENDOS rural supplies. In order to better monitor the water quality of rural supplies, an agreement was signed by the Ministry of Health and SENDOS in 1985. This act (SENDOS ORDINARY 1428/85) authorizes SENDOS to increase the sampling of drinking water to 75% in relation to NCh. 409 of 84 Standard Regulations.

Due to the high number of services to be controlled, their remoteness and inaccessibility to urban centres, the lack of rural laboratory facilities, and the lack of trained personnel and resources, SENDOS is unable to comply with the Act.

Available data indicate that only 63% of the necessary sampling is able to be accomplished (SENDOS, Informe Técnico Deprona, 1987).

On the basis of free chlorine availability data and coliform bacteria occurrence, the Ministry of Health affirms that urban drinking water is safe in Chile. Of the total urban services, 86% of them met the national bacteriological requirements in 1987. Considering that the urban Chilean population served by SENDOS is about 9.8 million, it can be assumed that 12.8% of the population could have been under a health risk by consuming unsafe water during 1987. Disinfection failures or the failure to use full conventional treatment, especially in small towns, are some of the causes of this situation. Also, natural disasters such as winter floods, earthquakes, and others, usually interrupt the normal drinking water supply with the result that water-borne diseases have occurred.

In rural areas, according to the special agreement, potable waters from SENDOS supplies appear to be good quality, but considering that only "two-bimonthly samples, collected for testing are both on the same day", data of these potable waters suggests users may be at some risk. In relation to the widespread rural population, there are no drinking water standards and no control of water is done, thus no data on the quality of water exist. Local studies undertaken by SENDOS have shown that the lack of potable water in these populations correlate to child diarrhoea morbidity in these rural areas. Another study performed close to Santiago city showed similar results (SENDOS, 1983; Castillo _et al_, 1984).

Very rarely are studies carried out in Chile in relation to quality of water. The most studied waters have been waste water and surface receiving waters, because of their use in agricultural irrigation. In general, waste water treatment is not practiced in Chile, and polluted waters are directly discharged into surrounding urban waters, which are used for multiple purposes. Thus, _Salmonella sp_. are commonly isolated from Chilean surface waters, especially in big cities (Castillo, G. _et al_, 1983; Campos, V. _et al_, 1986).

In relation to ground water quality, there are less data available than for surface water. Officials affirm that "because of its deepness, well water must be safe _per se_". However, faecal coliforms as high as 10,000/100 mL were detected in Chilean well waters from rural areas of Metropolitan Region. Nearby latrines, polluted surface water infiltration and soil porosity all contribute to the detriment of the water quality of Chilean well water (Castillo, G. _et al_, 1984).

Traditionally, APHA MPN tests are used by Chilean laboratories for monitoring bacteriological water quality. The APHA membrane filtration procedure has only been used since 1980. Problems with turbid waters and the high costs of the filtration units, membranes and dishes, are the main reasons for its scarce application in the country.

An important fact to be pointed out is that in Chile the monitoring for enteric viruses in water is not done as Chilean laboratories do not have the capability to perform these tests. Therefore, potable water safety is based on coliform indicator tests.

Thus, the Chilean situation strongly indicates that recent developments in simplified water quality testing procedures should be considered. Simplified tests such as the Presence/ Absence (P/A) test, the APHA faecal coliform A-1 assay, the hydrogen sulphide (H_2S paper strip) technique and coliphage assay (Clark, 1969; Andrews et al, 1972; Manja et al, 1982; Wetsel et al, 1982) would have great potential if they were used in Chile.

It would be necessary to evaluate the P/A, coliphage and H_2S tests in Chile for their applicability to screen drinking water quality, especially for improving the control of rural supplies.

The use of the faecal coliform A-1 assay and the coliphage test in Chilean waters would greatly assist in the speeding up of sanitary control of raw waters. In addition, since coliphage are more resistant to chlorine disinfection than coliform bacteria and they are viruses, they may be useful as indicators of disinfection efficiency and as enteric viruses tracers in drinking water (Setler, 1984; Martins, 1987).

In view of these needs, a study to evaluate these new methodologies of microbiological water quality control was done in Chile. The research was supported by the International Development Research Centre and the following objectives were established:

1. to evaluate rapid, inexpensive simple tests to determine the bacteriological quality of potable water sources and to produce criteria for classification of potable water sources; and,

2. to evaluate simple inexpensive bacteriological tests for screening drinking water.

Specific objectives were: to develop local expertise on coliphage, P/A and H_2S tests; to evaluate the ability of coliphage data to be used for classifying drinking water sources, by comparing the data to routine APHA, MPN, faecal coliform MF and APHA A-1 broth counts; to determine the specificity of A-1 broth, EC broth and MF MFC agar in relation to *E. coli* enumeration; to evaluate the relationship between coliphage and faecal coliform and to design criteria for classifying water sources in Chile; to evaluate P/A, H_2S and coliphage procedures as screening tests for drinking water, in comparison to conventional MPN and MF tests.

The results of the Chilean study are presented and discussed below.

METHODS

Water Samples

One hundred and twenty-six water samples were collected in triplicate from the following natural waters: river waters (42), creek waters (42), lagoons (19), drains (2), canals (2), urban wells (8) and rural wells (11). All of these are potable water sources from within the urban and rural areas of the Metropolitan Region. **Figure 1** shows the sampling sites and their respective nearby inhabited centres.

A total of 200 potable water samples were also assayed. The sources of these samples were: Santiago Metropolitan City piped water network (83) and rural distribution systems 117. Out of the 200 samples, 183 were collected from chlorinated systems and 17 from non-chlorinated wells or surface waters. Chlorinated samples were iced on collection and tested within six hours.

Bacteriological Test

Raw water samples were subjected to the following APHA Standard Methods (1985) total coliform and faecal coliform tests: the 5-tube MPN procedure using lauryl tryptose broth and brilliant green lactose bile broth with faecal coliform confirmation in EC broth; the 5-tube MPN procedure using A-1 broth (Difco No. 1823-17); and the membrane filtration procedure using M-Endo agar medium; and the 35°C heterotrophic spread plate count procedure using triptane glucose extract agar.

All drinking water samples were also tested by the Clark's Presence/Absence (P/A) test (1969), using rehydrated P/A medium (Difco No. 0019-17) and all positive tests were subjected to confirmation tests for total coliforms, faecal coliforms, faecal streptococci, _Clostridium_ _spp_., _Pseudomonas_ _aeruginosa_, _Stophylococci_ _aureus_ and _Aeromonas_ _spp_., as detailed by Clark _et al_ (1982). The drinking water samples were also tested by the hydrogen sulphide (H_2S) paper strip technique using chemically inoculated paper strips as described by Manja _et al_ (1982). All positive samples by the H_2S procedure were subjected to similar identification procedures as used in the P/A test.

Coliphage Test

This study used the procedure described by Wetsel _et al_ (1982) and reproduced in Section 919 C APHA Standard Methods (1985) with the addition of 2, 3, 5-triphenyl tetrazolium chloride and using _E_.

coli C, ATCC No. 13706 as host. All raw water samples and potable waters were coliphage assayed.

Physical and Chemical Tests

Turbidity measurements were made on the water samples using the nephelometric procedure and reporting results as NTU (APHA, 1985). Free residual chlorine was assessed in all chlorinated potable water samples using the APHA Standard Methods (1985) DPD colorimetric comparation method.

Biochemical Identification of Faecal Coliforms

A selected number of samples positive for faecal coliforms from MPN EC and A-1 tests, and MFC faecal coliform test, were subjected to E. coli identification procedures.

Usually positive MPN tubes (EC and A-1 broths) and typical and atypical MFC colonies were reisolated by streaking out onto MacConkey agar, the isolated colonies were subjected to the following tests: oxidase, IMVIC, ornithine and lysine decarboxylase, glucuronidase and growth in Kligler agar.

RESULTS

Raw Waters

All of the 126 raw water samples collected in triplicate were analyzed for total coliforms, faecal coliforms (three methods) and coliphage. Of the 126 samples tested, 118 were found to contain faecal coliforms. The A-1 broth test showed 105 polluted samples, 104 done by the EC broth and 103 done by the membrane filtration procedure.

Counts ranged from 2.0 to 9.2 x 10^5/100 mL for total coliform and for faecal coliform tests the ranges were: in A-1 broth from 2.0 to 5.4 x 10^4/100 mL; in EC broth from 2.0 to 7.0 x 10^4/100 mL; and on MFC agar from 1.0 to 4.0 x 10^4/100 mL.

It was observed that in samples having less than 10 faecal coliforms per 100 mL, negative results within one or two of the replicate samples were commonly found in all three tests used.

Data summary of total and faecal coliforms (three methods), and coliphages, from samples collected from different sources are shown in Table I. On the basis of total and faecal coliform tests, these data indicate that, with the exception of the Maipo River (which supplies water for the Vizcachas and Vizcachitas treatment plants) and some rural supplies, waters used for supplying drinking water in the Metropolitan Region are only minimally polluted.

In relation to three different methods used to quantify faecal coliforms, it was observed that they were similarly sensitive for detecting faecal pollution indicator organisms. Usually the densities obtained by the three procedures had the same order of magnitude (Table I).

Statistical regression analysis of 363 data points indicated a good correlation between the three methods. Correlations found were: EC/A-1 = 0.82; EC/MFC = 0.77; MFC/A-1 = 0.74.

Data correlation equations obtained were:

$$\log \text{EC} = 0.164583 + 0.885849 \log \text{A-1} \quad (r\ 0.82;\ n.\ 363)$$

$$\log \text{EC} = 0.271157 + 0.889348 \log \text{MF} \quad (r\ 0.77;\ n.\ 363)$$

$$\log \text{MFC} = 0.067892 + 0.832790 \log \text{A-1} \quad (r\ 0.74;\ n.\ 363)$$

These data are consistent and show that the A-1, 24-hour procedure could be used in Chile instead of the conventional, time-consuming and more expensive EC test, or the most expensive test, the MFC, for classifying natural water sources for drinking water purposes, or other uses such as irrigation, recreation, etcetera.

In addition, this study also demonstrated the selectivity of the A-1 test for *E. coli* detection. From 91 positive samples in A-1 medium, *E. coli* was recovered in 89 samples. In two "false positive" A-1 samples, *E. aerogenes* (1) and *Aeromonas spp.* (1) were isolated. However, of the 893 isolates collected from the 89 samples, 92% of them were biochemically identified as *E. coli* The other bacteria were *E. aerogenes*, *Citrobacter spp.*, *E. agglomerans*, *Aeromonas spp.* and other fermenter and non-fermenter bacteria.

On the other hand, from 76 reisolated positive samples in EC broth, all had *E. coli*, and in 89.1% of 854 subcultures submitted to biochemical tests, *E. coli* was found. Other selected bacteria were identified as *E. aerogenes*, *Citrobacter spp.*, *Aeromonas spp.*, *Proteus spp.*, *Ps. aeruginosa* and some non-identified bacteria by the biochemical tests used.

Ninety-one samples containing various types of blue colonies growing on MFC agar, which were counted as faecal coliforms, were tested for *E. coli* and only in three samples *E. coli* bacteria were not found. Strains that gave false positive results were identified as *Aeromonas spp.* in two samples and *Enterobacter agglomerans* in the other sample. A total of 609 isolates of typical blue colonies from 89 samples were submitted to biochemical assay and only 72.9% of them were found to be *E. coli*, *E. agglomerans*, *Citrobacter spp.*, *E. aerogenes*, *Aeromonas spp.*, Klebsiella spp., Proteus spp., Edwardsiella and a great number of non-identified strains made up the rest of the isolates collected for identification.

On MFC agar, brilliant blue or opaque dark and light blue colonies were often found mixed among the typical blue faecal coliform colonies. These colonies, which added confusion when counting colonies, were identified predominantly as Klebsiella and Pseudomonas spp. and others not able to be identified, both fermenters, and non-fermenters. It was usually noted that highly polluted waters had more interfering bacteria on MFC agar than clean waters. Other atypical colonies on MFC agar were grey, pink and translucid colonies.

Based on the faecal coliform isolates from raw water, the A-1 broth technique was found to be the most selective procedure for E. coli detection, with the MFC test being the least selective. These observations reaffirm the advantage for using the A-1 procedure for classifying all natural waters in Chile, because of its good correlation to conventional tests and its excellent selectivity to detect the faecal pollution indicator, E. coli.

The coliphage test was applied to 126 triplicate raw water samples and in 63 of them coliphage were found in at least one of the replicates. Coliphage counts in triplicate samples showed great variation within the replicates, so sometimes all replicates produced the same results and variations as high as 300% or more were also found. Summarized data of the geometric mean coliphage counts from the different water sources tested are shown in Table I.

These data indicate that in waters containing low faecal pollution indicator counts, usually low numbers (or no) of coliphages were found.

Coliphage densities in waters ranged from 5 to 1,250 PFU/100 mL, with an average of 0.6 PFU/100 mL. Regression analysis of the data showed no correlation between coliphage and faecal coliform counts (three methods) in these raw waters.

Despite this disagreement, an interesting relation was observed with water turbidity. Table II shows faecal coliforms (EC broth) and coliphage mean values from different sampling sites related to water turbidity ranges. These data indicate that usually in most of the clean and slightly faecally polluted waters, coliphage recovery was usually related to the degree of faecal pollution (FC counts) in the waters (for example, rural wells, Laguna Negra). But when turbidity and faecal pollution (FC counts) of water increased, irregular coliphage results were obtained (for example, La Turca Canal, Maipo River). Based on these data and on data reported by Oghaki (1986), we can assume that, due to the capability of coliphage to be absorbed on particulate materials, turbidity of water is an important parameter to be considered when using the coliphage test as a tool for classifying raw waters in Chile.

In order to obtain further information about coliphage behaviour in relation to water turbidity, 60 positive coliphage samples were classified according to three different water turbidity patterns (**Tables III to V**).

Table III presents data from 21 samples containing coliphages, in at least one of their replicates, in water with turbidity levels of ≤15 NTU. Also, faecal coliform densities obtained by three methods are shown. In these samples, the average coliphage count was 15.1 PFU/100 mL and usually the same order of magnitude counts were found within the replicates. It was observed that in 16 samples having low faecal coliform counts (EC av. 7/100 mL) no coliphages were found in most of the replicates; however, in 6 samples where faecal coliforms were absent, coliphages were recovered. These waters were in the mountains where low human activities exist. Poor correlations between faecal coliforms (three methods) and coliphage were found in these waters (EC = 0.39, MFC = 0.37; A-1 = 0.26) and there were no correlations with water turbidity.

Based on these results and unpublished data, we believe that the failure to recover coliphage from these and other waters is more related to the low sensitivity (20 mL examined only) of the coliphage technique rather than water turbidity.

Data of **Table IV** show coliphage recovery from 18 triplicate samples where water turbidity ranged from 22 to 71 NTU. Coliphage counts in these waters fluctuated from 5 to 480 PFU/100 mL with a geometric mean of 25 PFU/100 mL. In these samples, better coliphage recovery within replicates was obtained and also low variations between coliphage counts occurred.

Correlations among faecal coliforms (three methods) to coliphage in these medium turbid waters were better than in waters with low turbidity, for example, A-1 = 0.59, MFC = 0.55, EC = 0.44, respectively. Again, no correlation between water turbidity and coliphage was found.

In 21 triplicate samples containing turbidity levels ranging from 127 to 800 NTU, coliphage counts fluctuated from 5 to 1,250 PFU/100 mL (**Table V**), with a mean of 24 PFU/100 mL. The mean faecal coliform density of the samples were 535 MPN-EC/100 mL, and no correlation between turbidity and faecal coliforms to coliphage was found (MFC = 0.23, A-1 = 0.19, EC = 0.12).

We want to point out that most of these samples were collected from the same sites which had medium turbidity levels, but in different seasons (Tables IV and V). If we compare coliform levels of these samples, we can see they are similar.

Great variations in turbidity and hardness levels of surface water are common in Chile, especially in late Spring and Summer; hardness as high as 500 mg/L CaCO3, can be found; and turbidities as high as 1,200 NTU were noted. This phenomenon is due to the melting of ice on the Andes Mountains. We believe the irregular coliphage recoveries found in these waters is probably related to seasonal variations in both hardness and turbidity.

Based on these data and on studies reported by Oghaki et al (1986) which demonstrated the effect of bi-valent cations (Ca and Mg) as coliphage removers from water, we have started studies to elucidate coliphage behaviour in high turbidity and hard Chilean raw waters.

The results from this study are still in the preliminary processing stage and will be available in the future.

Drinking Water

A total of 200 drinking water samples (non-replicated) were collected from the Santiago city piped network and public and private distribution systems, as well as from rural individual supplies. One hundred and eighty-three samples were collected from chlorinated systems and 17 were from non-chlorinated wells.

Summarized data from the 200 drinking water samples collected from different sources, as well as the tests applied, are shown in **Table VI**. On the basis of MPN coliform results, it can be noted that urban drinking water showed better sanitary quality than rural chlorinated and non-chlorinated potable water.

One hundred and fifty of the 183 samples collected from chlorinated systems had free residual chlorine levels ranging from 0.1 to 0.7 mg/L.

Of the 200 samples tested, 58 were found to contain undesirable bacteria in at least one of the tests used. Coliphage were also detected in five samples.

Of these 58 unsafe samples, 33 had coliforms and showed P/A and H_2S paper strip (20°C and 35°C) positive tests. Frequency distribution of positive samples, in relation to their source were: non-chlorinated supplies (wells) (100%), private rural systems (41.9%), public rural systems (33.9%) and urban systems (4.8%).

The P/A test was the most sensitive, producing 54 (27%) positive tests on the 200 samples, followed by total coliforms (two methods) with 42 (21%) positive tests, and by the H_2S assay, both temperatures, with 32 (16%) positive tests. Faecal coliforms were also detected in 26 (13%) drinking water samples.

The comparison of the methods in detecting the presence of unsafe waters, from 41 positive chlorinated samples (in at least one of the tests used), are displayed in **Table VII**. Free residual chlorine and Heterotrophic Plate Count (HPC) data are also included. In the P/A test, 37 samples were positive, followed by coliforms (26 and 25), and H_2S test (16). Coliphage were recovered from five samples, and HPC were high with an average of 550 CFU/mL.

From P/A data, some interesting findings were noted. The four samples that were negative by this test had coliform densities by at least one of the three methods of <1, 1, 2 and 3/100 mL; in 9 samples, containing free residual chlorine, the P/A test was the only bacterial indicator test positive in these waters (**Table VII**).

In samples positive by the H_2S test, coliforms were present in 16 of the 17 samples. However, 9 samples with coliforms were H_2S test negative. Coliform counts in these samples (except one), were low (<8/100 mL). Due to differences in sample volumes tested by the different methods (20 mL in H_2S test and 55.5 to 100 mL in the MPN and MF procedures, respectively), the differences in sensitivities may be due to volume variations. It was also noted that incubation temperature influenced results observations. For instance, H_2S tests which were positive after 24-hours incubation at 35°C were usually positive in 48 hours when incubated at 20°C (**Table VII**).

Data from well water are displayed in **Table VIII**. It can be seen that all of the 17 samples tested were contaminated. The heterotrophic plate count (HPC) ranged from 110 to 54.000 mL, total coliforms fluctuated from 13 to 1,600/100 mL, and faecal coliforms ranged from 2.0 to 920/100 mL in these samples.

Again, the P/A test was the most sensitive indicator with all 17 samples being P/A positive. The MF coliform test was positive in 16 samples; the H_2S 20°C and total coliform MPN tests were positive in 14 samples; while the H_2S 35°C test was positive in 13 samples. Faecal coliforms were present in 11 samples.

The only P/A positive sample, where no coliforms were detected, also had a high HPC (330 CPU/mL) count. Similar to chlorinated waters, H_2S negative samples had low coliform counts (<13/100 mL).

Again, accordance between H_2S positive tests, in relation to incubation temperature and time for positive results to appear, was obtained.

Based on these data, we believe that P/A and H_2S (20°C and 35°C) tests would be useful for controlling potable water in Chile. The P/A test has the advantage of being more simple and sensitive than conventional MPN and MF procedures, and shows good agreement with both of these tests in monitoring the safety of potable waters. The H_2S test also proved to be an adequate procedure for detecting unsafe drinking water and, due to its simplicity and feasibility of being carried out at room temperature, it would be a good tool for screening potable water in rural areas under field conditions.

In order to determine the selectivity of the P/A and H_2S tests for detecting coliform bacteria and other indicators in drinking water, a study to validate both methodologies was also done. Thus, the biochemical identification of organisms growing in all positive P/A and H_2S (20°C and 35°C) tests was done. Bacterial identification by APHA (1985), and other additional tests, were carried out in the 54 P/A positive samples and in the 61 (20°C and 35°C) positive H_2S tests.

Out of the 54 P/A positive samples, coliform bacteria were confirmed in 48 samples. *E. aerogenes*, *E. agglomerans*, *E. coli*, *E. cloacae*, *Citrobacter spp.* and *Klebsiella spp.* were found. Other bacteria growing in P/A positive tests were *Clostridium spp.* (32), faecal *streptocci* (21), *Pseudomonas spp.* (9) and *Aeromonas spp.* (6). No *S. aureus* was detected in these samples.

Out of the six P/A positive samples, where no coliforms were recovered, *Clostridium spp.* and *Pseudomonas spp.* were detected.

In isolates from 61 H_2S positive tests, coliforms were usually confirmed, the most common being *Citrobacter spp.* Some H_2S producing *E. coli* were also identified. Other H_2S producing organisms found were *Clostridium spp.*, *Proteus spp.* and *Providence spp.* Typical non H_2S coliforms such as *E. agglomerans*, *E. coli*, *E. aerogenes* and *Klebsiella* were also identified. *Aeromonas spp.*, *Pseudomonas spp.* and other non-identified strains were also detected in H_2S positive samples. Usually bacteria developing at 20°C were similar to those growing at 35°C.

All H_2S positive tests were also tested for *Salmonella spp.* presence. Isolations on bismuth-sulphite agar, and Kauffman BPL agar were done, but these pathogenic bacteria was never recovered. The selectivity, with regards to coliform presence, showed by P/A and H_2S tests, is another quality of these procedures which, together with the sensitivity presented above, convinced us that both tests would be of great use for controlling the sanitary quality of water in Chile. We believe that P/A test could perfectly replace conventional Chilean bacteriological potable water quality tests, with the added advantage of detecting both coliforms and other indicators. Also, the adoption of the H_2S test as a routine practice in remote areas would be a real contribution to improving the potable water quality of Chilean rural supplies.

In relation to coliphage assay, coliphage were detected in five samples collected from the same source. This supply was a chlorinated rural system, but in samples containing coliphages, residual chlorine was absent. Coliphage range of samples was from 10 to 115 PFU/100 mL, with faecal coliforms ranging from 13 to 350/100 mL. The relation found between faecal coliforms to coliphage was 1.18:1.

An important fact to be noted is that when free chlorine was available, no coliphages were detected. As we observed earlier in this study, coliphages were present in raw water sources and after these waters have been treated, no coliphages have been recovered.

On the basis of these findings, and other local experiences developed in the IX Region of the country where usually coliphage counts in water source are about 200 PFU/mL, and after treatment they are not found, we assume that phages are being eliminated by the conventional drinking water treatment used in Chile (Castillo, G., 1988).

Our data would be in accordance with reports that indicate no coliphage detection after full water treatment (Stetler, 1984; Martins, 1987).

For these reasons, we believe that coliphages should be used for monitoring potable water in Chile, especially in localities where raw waters are recipient of waste waters. As it was mentioned above, waste water are not treated in the country, no viruses monitoring is performed, and viral pathologies are endemic.

Therefore, in our opinion, the P/A test combined with coliphage test would guarantee the safety of drinking water from both pathogenic bacteria and enteric viruses infections in Chile.

DISCUSSION

The evaluation of simple, rapid and inexpensive bacteriological methods for classifying potable water sources in Chile was the first objective of this IDRC-supported study. In this context, the study demonstrated that the A-1 faecal coliform test met these characteristics. It was very simple to operate, and the results were obtained within 24 hours.

Cost-effective studies indicate that the A-1 test (using commercial dehydrated media) cost CLP $320 for media alone per sample tested while the cost of traditional Chilean MPN and membrane filtration tests average CLP $280 and $200, respectively, for media and membranes. However, when the two sets of MPN media and process steps and the up-to-five days required for final results by the MPN procedure are compared to the single media and one-process step and 24-hour results of the A-1 broth procedure, we conclude that the cost-effectiveness between these two tests are compensatory. Also, the costs of the A-1 broth can be lowered by making the media by chemical formulation.

Even though the estimated cost of obtaining MF results per sample (based on one filtration) was the lowest, the high cost of the filtration equipment and the foreign dependence on membrane filter and petri dish supplies must be taken into account. Furthermore, technical difficulties with MF counts/colony interpretation which occurred in the study, encourages us to recommend the A-1 broth test over the MF test, even though final results are produced within 24 hours by both procedures.

Good linear correlation between A-1 test and conventional EC broth, and membrane filtration m-Endo LES test data, was found in this study. Also, non-parametric statistical analysis for rank correlation of data from 78 triplicate water samples showed a positive and significant (<.01) association between the three tests. That means, the quality of Chilean water sources can be characterized by any of the methods (**Table IX**).

The selectivity of the A-1 medium in this study was consistent and reaffirms its use for detecting faecal pollution in water.

In **Table IX**, it can be seen that using Spearmans Rank Correlation test that the coliphage test was significantly correlated (p <.01) to all three faecal coliform enumeration procedures, but not to the total coliform count procedure.

Physical and chemical factors such as flow rate, turbidity, hardness, and probably other unknown factors, may contribute to irregular coliphage recovery in Chilean natural waters. However, because of coliphage, counts are rapid (obtainable within 4 to 6 hours) and are simple to perform, they are being proposed as indicators of insufficient disinfection and faecal pollution; further studies must be carried out in order to decide whether this test should be used for classifying potable water sources for drinking water in Chile.

As the second objective of this study, the simplicity, economy and effectiveness of the P/A and the H_2S methods for screening drinking water in Chile were clearly demonstrated.

In both tests only one bottle containing culture media was needed to test the water; they can be carried out to the field, so samples could be inoculated directly on site. No problems with sample processing or data interpretation were observed, and both activities can be easily done by untrained personnel.

Cost-effective studies carried out show that both tests would be very inexpensive if used in Chile. The P/A test cost, including disposable bottle, would be CLP $180, while the H_2S test would be only CLP $35 (bottle included also). That means both tests would be less expensive than the MPN and MF tests presently used in Chile.

Because of their sensitivity and selectivity, P/A and H_2S tests were very effective in detecting pollution in potable water, especially the P/A test which, in several opportunities, was the only test to demonstrate the presence of bacterial indicators. Also, its wide spectrum for detecting other indicators such as faecal streptococci, Clostridium, Pseudomonas and Aeromonas makes this method more sensitive than conventional methods and strongly suggests that this procedure be used for screening sanitary quality of potable water in Chile.

Considering the poor quality of rural potable water supplies shown by this study and the scarcity of water quality testing that is being done to rural services in Chile, the H_2S test would be the most appropriate mechanism to be adopted for solving both problems.

Although the H_2S test is easy to perform and can be done under field conditions, Chile's widely differing climatic conditions suggest more information is required to establish temperature requirements of the test. Also, since the present sample volume is 20 mL, studies on the sensitivity and selectivity of the test using larger volumes of water would be in order, especially for use in potable waters with little or no pollution.

In our study, the coliphage test did not produce many positive findings in non-treated potable waters. However, the data suggest that it was worthwhile to include the coliphage test to reinforce potable water quality estimates, especially in areas where water sources were polluted. We believe that all of the objectives of the IDRC-supported study have been fully met.

The next step in this program is to obtain recognition by government officials and laboratory personnel of the potential of these tests to classify potable water sources and for testing drinking water safety.

CONCLUSIONS AND RECOMMENDATIONS

On the basis of data obtained in this study and their discussion, we conclude the following:

1. A-1 faecal coliform test, due to its simplicity, sensitivity, specificity, good correlation with conventional tests, prompt results and fair cost, it could advantageously be used to replace the MPN or membrane filtration methods for classifying potable water sources in Chile.

2. The Presence/Absence (P/A) test and the Hydrogen-Sulphide (H_2S) test, because of their simplicity, very cheap costs and good accordance to total coliform indicator and other related indicators, could be recommended as good alternative tools for screening water safety of potable water in Chile.

3. The P/A test could become a convenient method to be adopted, instead of conventional tests, especially for controlling drinking waters in urban areas where a high number of samples must be collected. The H_2S test should be considered as a means for improving both safety and quality control of potable water in Chilean rural supplies.

4. Considering that very different weather conditions and high seasonal variations on environmental temperatures occur throughout the country, the optimal operational conditions of the H_2S test must be determined before adopting it for qualifying potable water in Chilean rural areas.

5. As a model for tracing viruses and as indicators of drinking water treatment disinfection efficiency, coliphages should be taken into account in this country, especially at sites where water sources are recipients of waste waters and enteric viruses could be present.

6. Further studies must be carried out in Chilean surface waters in order to determine whether factors such as physical and chemical quality of water and others would have influence on coliphage persistence in water.

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TABLE I

SUMMARY DATA OF 126 TRIPLICATE RAW WATER SAMPLES FROM DRINKING WATER SUPPLIES OF THE METROPOLITAN REGION OF CHILE (1986-1987)

Water Source	Total Coliforms MPN/100 ml		Fecal Coliforms/100 ml						Coliphage PFU/100 ml	
			MPN EC broth		MPN A-1 broth		mFC agar			
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Arrayán*	218	22-1600	17	< 2.0-240	20	< 2.0-130	15	< 1-58	0.15	< 5-10
Drains*	78	22-350	16	7.8-33	24	6.8-49	15	6-36	0	-
El Canelo*	174	2.0-1600	1.5	< 2.0-79	0.9	< 2.0-23	0.6	< 1-56	0.6	< 5-10
El Manzano*	197	2.0-1600	3.0	< 2.0-33	2.2	< 2.0-13	1.6	< 1-20	0.8	< 5-85
La Dehesa*	77	4.5-540	2.5	< 2.0-240	1.4	< 2.0-170	5.0	< 1-104	0.2	< 5-15
Laguna Negra*	115	4.5-1600	1.1	< 2.0-14	0.8	< 2.0-13	0.8	< 1-15	0.2	< 5-20
Las Perdices*	4005	1700-7000	88	68-130	58	49-79	59	50-75	1.4	< 5-5
Maipo*	1350	210-5400	450	140-1300	471	230-2400	243	< 1-1800	2.7	< 5-300
Mapocho*	302	14-1600	41	< 2.0-1600	130	4.0-1600	8.1	< 1-216	0.1	< 5-5
San Ramón*	168	49-920	3.7	2.0-7.8	2.6	< 2.0-13	4.8	2-100	0	-
Vizcachas and Vizcachitas*	1664	170-16000	561	49-2400	541	78-2400	152	< 1-4000	3.2	< 5-1250
Urban wells*	4.6	< 2-79	0.2	< 2.0-7.8	0.2	< 2.0-7.8	0.2	< 1-12	0	-
El Clarillo**	2009	240-16000	1018	110-3500	606	< 2.0-5400	439	20-2250	29	< 5-480
Juan Fernández**	363	170-920	124	33-350	132	49-220	48	6-165	0	-
La Turca**	19000	7000-92000	4837	3300-7000	4206	3300-4900	3634	3000-4000	20	50-150
Rural wells**	521	33-16000	1.6	< 2.0-240	0.9	< 2.0-240	0.9	< 1-140	0.3	< 5-20
	272	< 2.0-92000	17	< 2.0-7000	16	< 2.0-5400	10	< 1-4000	0.6	< 5-1250

* Urban drinking water source

** Rural drinking water source

TABLE II

SUMMARY DATA OF TURBIDITY RANGE, EC BROTH FAECAL COLIFORMS AND COLIPHAGE MEANS OF CHILEAN RAW WATER SUPPLIES FOR DRINKING WATER (1986-1987)

Source	Turbidity range (NTU)	Faecal Coliforms EC broth MPN/100 mL (geom.aver.)	Coliphages PFU/100 mL (geom.aver.)
Urban wells	1.0	0.2	<5
Rural wells	2.0	1.6	0.9
Drains	2.0	1.6	<5
Laguna Negra	5.0	0.1	0.2
San Ramon	5.0	3.7	<5
El Canelo	12	1.5	0.6
El Manzano	13	3.0	0.8
La Dehesa	15	2.5	0.2
El Clarillo	15	1016	29
Las Perdices	25	88	1.4
Arrayan	50	17	0.2
Mapocho	60	41	0.1
La Turca	100	4837	20
Vizcachas/Vizcachitas	200	561	3.2
Maipo	350	450	2.7

TABLE III

POSITIVE COLIPHAGE TRIPLICATE SAMPLES FROM LOW TURBIDITY (≤15 NTU) CHILEAN WATER SOURCES

No.	Source	Turbidity NTU	Faecal Coliforms/100mL			Coliphages CFU/100 mL
			EC Broth	A-1 Broth	MFC	
3a	La Dehesa	10.5	<2.0	<2.0	10	15
6b	La Dehesa	11.0	<2.0	<2.0	3	5
10a	La Dehesa	10.5	<2.0	<2.0	5	5
10b			<2.0	<2.0	2	5
8a	Mapocho	14.0	<2.0	33	0	5
28b	El Manzano	3.0	23	13	6	5
34	El Manzano	2.4	6.8	13	11	55
34a			13	4.5	20	55
34b			13	4.5	-	50
40	El Manzano	0.9	17	13	6	15
40a			13	13	12	30
40b			23	2.0	20	65
46	El Manzano	1.8	13	13	20	5
46a			13	13	10	85
46b			33	2.0	6	5
52b	El Manzano	1.3	6.8	<2	0	5
64a	El Manzano	0.7	2.0	4.5	1	5
64b			7.8	4.5	0	10
29a	El Canelo	3.0	<2	<2	0	10
65a	El Canelo	1.0	4.5	23	0	5
30	Laguna Negra	5.0	7.8	<2.0	2	10
30b			2.0	<2.0	14	20
36	Laguna Negra	5.0	2.0	4.0	2	5
36b			2.0	2.0	4	5
42	Laguna Negra	3.7	4.5	4.5	2	5
54a	Laguna Negra	9.9	4.0	<2.0	0	10
113	Las Balsas	1.2	110	240	70	5
113a			240	130	140	15
113b			79	49	100	20
114	El Paraiso	4.5	14	49	60	5
124	El Clarillo	1.5	390	540	580	60
124a			3,500	1,600	410	400
124b			2,200	1,700	290	120
125	El Clarillo	4.5	1,300	1,600	400	75
125a			2,400	920	267	35
125b			1,700	540	100	35
126a	El Clarillo	5.1	240	170	40	15
126b			350	-	100	20

TABLE IV

POSITIVE COLIPHAGE TRIPLICATE SAMPLES FROM MEDIUM TURBIDITY (22-71 NTU) CHILEAN WATER SOURCES

No.	Source	Turbidity NTU	Faecal Coliforms/100mL			Coliphages CFU/100 mL
			EC Broth	A-1 Broth	MFC	
20B	Mapocho	35	24	1,600	170	5
84	Las Pérdices	25	78	49	50	5
84a			130	79	55	5
96a	Maipo	28	490	330	200	20
96b			140	230	300	20
100	Maipo	45	490	490	200	20
100a		45	790	490	100	5
97	Vizcachas	30	220	490	100	5
101	Vizcachas	41	330	330	300	5
105	Vizcachas	50	790	950	800	5
105a			790	330	1,000	25
105b			2,400	490	1,200	5
109	Vizcachas	32	130	78	180	15
98	Vizcachitas	30	790	130	100	5
98a			490	790	200	5
98b			490	490	400	25
102a	Vizcachitas	41	1,700	490	300	5
102b			790	490	500	15
106	Vizcachitas	50	1,100	330	800	10
106a			1,300	490	300	15
106b			490	490	400	10
110	Vizcachitas	46	49	130	100	5
110a			79	78	30	5
117	El Clarillo	71	2,800	1,600	1,200	90
117a			3,500	1,700	2,200	145
117b			2,400	3,500	1,500	285
118	El Clarillo	46	1,700	1,600	1,200	385
118a			1,600	920	1,600	380
118b			3,500	3,500	900	440
119	El Clarillo	46	130	240	220	5
121	El Clarillo	56	2,400	2,400	1,200	415
121a			1,300	2,400	1,800	330
121b			3,500	3,500	2,250	480
122	El Clarillo	22	1,800	2,400	550	180
122a			790	3,500	900	170
122b			2,400	5,400	1,000	200
123a	El Clarillo	50	350	240	600	5

TABLE V

POSITIVE COLIPHAGE TRIPLICATE SAMPLES FROM HIGH TURBIDITY (126-800 NTU) CHILEAN WATER SOURCES

No.	Source	Turbidity	Faecal Coliforms/100mL			Coliphages CFU/100 mL
			EC Broth	A-1 Broth	MFC	
25	Arrayan	137	49	22	24	10
25a			10	17	10	5
31	Maipo	800	1,300	1,700	900	100
31a			1,300	2,400	1,800	100
31b			1,300	1,100	1,200	300
37	Maipo	275	220	790	1,200	100
37b			260	490	500	100
43	Maipo	420	490	790	1,000	40
43b			490	790	600	25
49a	Maipo	128	490	230	300	15
55	Maipo	690	490	230	200	5
55a			490	230	200	20
55b			700	230	300	10
67	Maipo	310	330	790	300	25
67a			490	230	200	10
67b			330	330	300	15
32	Vizcachas	800	330	1,100	1,400	100
32b			330	1,100	1,900	150
44	Vizcachas	415	330	790	700	15
44a			490	790	180	15
50	Vizcachas	135	79	1,300	900	20
50a			1,100	1,700	200	15
50b			1,400	790	900	10
56	Vizcachas	710	230	230	500	20
56a			1,700	1,300	1,000	30
56b			700	1,700	1,000	15
62a			330	490	1,000	20
68	Vizcachas	300	330	1,100	400	5
68a			230	1,300	600	20
68b			490	1,100	200	10
74	Vizcachas	410	790	790	500	10
74b			330	1,300	200	15
33	Vizcachitas	800	1,700	1,300	1,100	1,250
33a			1,300	1,420	400	400
33b			790	700	600	100
39	Vizcachitas	300	330	790	1,000	5
45a			330	790	1,000	15
45b			390	330	1,000	10
51	Vizcachitas	127	790	790	1,000	5
51b			490	700	400	5
57	Vizcachitas	700	2,400	1,100	1,000	15
57b			330	790	300	15
68	Vizcachitas	320	2,400	490	400	10
68b			1,300	490	200	10
81	La Turca	130	7,000	4,600	3,000	50
81a			4,900	4,900	4,000	150

TABLE VI

COMPARISON OF COLIFORM DETECTION BY P/A, H_2S, MF AND MPN METHODS

Number of Samples

Source of Samples	Total of Samples Per System	Free Residual Chlorine (+)	Total Samples (+)	Total Simultaneous* (+)	P/A (+)	H_2S (+)		Total Coliforms (+)		Faecal Coliforms (+) MPN	Coliphage (+)
						20°C	35°C	MF	MPN		
Chlorinated Supplies											
Urban Systems	84	83	4	1	4	1	1	1	3	2	0
Public Rural Systems	56	46	19	7	16	5	4	12	10	1	0
Private Rural Systems	43	21	18	11	17	11	12	13	15	12	5
Chlorinated Sub Total	183	150	41	19	37	17	17	26	28	15	5
Not Chlorinated Supplies	17		17	14	17	14	13	16	14	11	0
Total For Study	200	150	58	33	54	31	30	42	42	26	5
%		82.0	29.0	16.5	27.0	15.5	15.0	21.0	21.0	13.0	2.5

* Samples simultaneously (+) In: P/A, H_2S, and TC (MPN or FM) procedures.

TABLE VII: CHLORINATED DRINKING WATER - SUMMARY OF POLLUTED SAMPLES

Number of samples examined: 183 Number of positive samples: 41

Sample No.	Free Residual Chlorine mg/L	P/A (day)	"H_2S" test (day) 20°C	"H_2S" test (day) 35°C	TC mEndo MF	TC MPN	FC MPN	Coliphage	Heterotrophic counts per mL
1	0.00	+(1)	+(2)	+(1)	+	+	+	–	1,700
2	0.10	+(1)	+(2)	+(1)	+	+	+	–	3,350
8	0.00	+(1)	+(2)	+(1)	+	+	+	–	6,100
18	0.20	+(1)	–(5)	–(5)	–	+	+	–	130
27	0.40	–(5)	–(5)	–(5)	–	+	–	–	2
44	0.30	+(4)	–(5)	–(5)	–	–	–	–	25
45	0.00	+(1)	+(2)	+(1)	+	+	+	+	800
46	0.00	+(1)	+(2)	+(1)	+	+	+	–	1,200
47	0.00	+(1)	+(2)	+(1)	+	+	+	+	700
48	0.60	–(5)	–(5)	–(5)	+	–	–	–	1
52	0.00	+(1)	+(2)	+(1)	+	+	+	+	750
53	0.00	+(1)	+(2)	+(1)	+	+	+	+	920
54	0.00	+(1)	+(2)	+(1)	+	+	+	+	1,300
55	0.00	+(1)	+(2)	+(1)	+	+	+	–	770
56	0.00	+(1)	–(5)	+(1)	+	+	+	–	130
57	0.00	+(1)	+(2)	+(1)	+	+	+	–	30
60	0.00	+(3)	–(5)	–(5)	–	–	–	–	140
68	0.00	+(5)	+(4)	–(5)	–	+	+	–	10
79	0.00	+(3)	+(4)	+(3)	+	–	–	–	1
95	0.00	+(1)	–(5)	–(5)	+	+	–	–	30
111	0.30	–(5)	–(5)	+(5)	–	–	–	–	<1
120	0.40	+(4)	–(5)	–(5)	–	–	–	–	1
140	0.20	+(1)	+(4)	+(4)	+	+	+	–	1,000
142	0.00	+(2)	+(5)	–(5)	+	+	–	–	140
145	0.20	–(5)	–(5)	–(5)	+	+	+	–	3
155	0.20	+(2)	–(5)	–(5)	+	–	–	–	32
157	0.00	+(1)	–(5)	+(1)	+	+	–	–	200
158	0.00	+(4)	–(5)	–(5)	–	+	–	–	500
162	0.70	+(4)	–(5)	–(5)	–	+	–	–	2
164	0.00	+(3)	+(5)	–(5)	+	+	–	–	34
167	0.70	+(4)	–(5)	–(5)	–	–	–	–	60
179	0.00	+(1)	–(5)	–(5)	+	+	–	–	900
180	0.00	+(3)	–(5)	–(5)	+	+	–	–	1,300
183	0.40	+(5)	–(5)	–(5)	–	–	–	–	6
184	0.40	+(5)	–(5)	–(5)	–	–	–	–	2
185	0.20	+(5)	–(5)	–(5)	–	–	–	–	34
187	0.40	+(4)	–(5)	–(5)	–	–	–	–	38
190	0.40	+(1)	–(5)	–(5)	+	–	–	–	<1
195	0.20	+(3)	–(5)	–(5)	–	–	–	–	32
196	0.10	+(3)	–(5)	–(5)	–	–	–	–	1
198	0.20	+(2)	+(3)	+(2)	+	+	–	–	170
OTAL	20	37	17	17	25	26	16	5	

TABLE VIII

WELL WATER - SUMMARY OF POLLUTED SAMPLES

Number of samples examined: 17
Number of samples positives: 17

Sample No.	P/A (day)	"H_2S" test (day)		TC mEndo MF	TC MPN	FC MPN	Coliphage	Heterotrophic counts per mL
		20°C	35°C					
4	+(1)	+(2)	+(1)	+	+	+	−	5,800
5	+(1)	+(2)	+(1)	+	+	+	−	1,500
6	+(1)	+(1)	+(1)	+	+	+	−	33,000
7	+(1)	+(1)	+(1)	+	+	+	−	54,000
9	+(1)	+(2)	+(1)	+	+	+	−	680
71	+(1)	+(2)	+(1)	+	+	+	−	700
72	+(1)	+(2)	+(1)	+	+	+	−	1,400
73	+(1)	+(2)	+(1)	+	+	+	−	2,000
74	+(1)	+(2)	+(1)	+	+	+	−	16,000
75	+(2)	+(2)	+(1)	+	+	−	−	4,000
77	+(1)	+(2)	+(1)	+	+	+	−	2,100
131	+(3)	−(5)	−(5)	+	+	−	−	110
133	+(3)	−(5)	−(5)	−	−	−	−	330
139	+(5)	−(5)	−(5)	+	−	−	−	1,160
147	+(2)	+(5)	+(1)	+	+	−	−	350
150	+(4)	+(5)	−(5)	+	−	−	−	580
169	+(1)	+(2)	+(1)	+	+	+	−	>1,000
TOTAL +	17	14	13	16	14	11	0	

TABLE IX

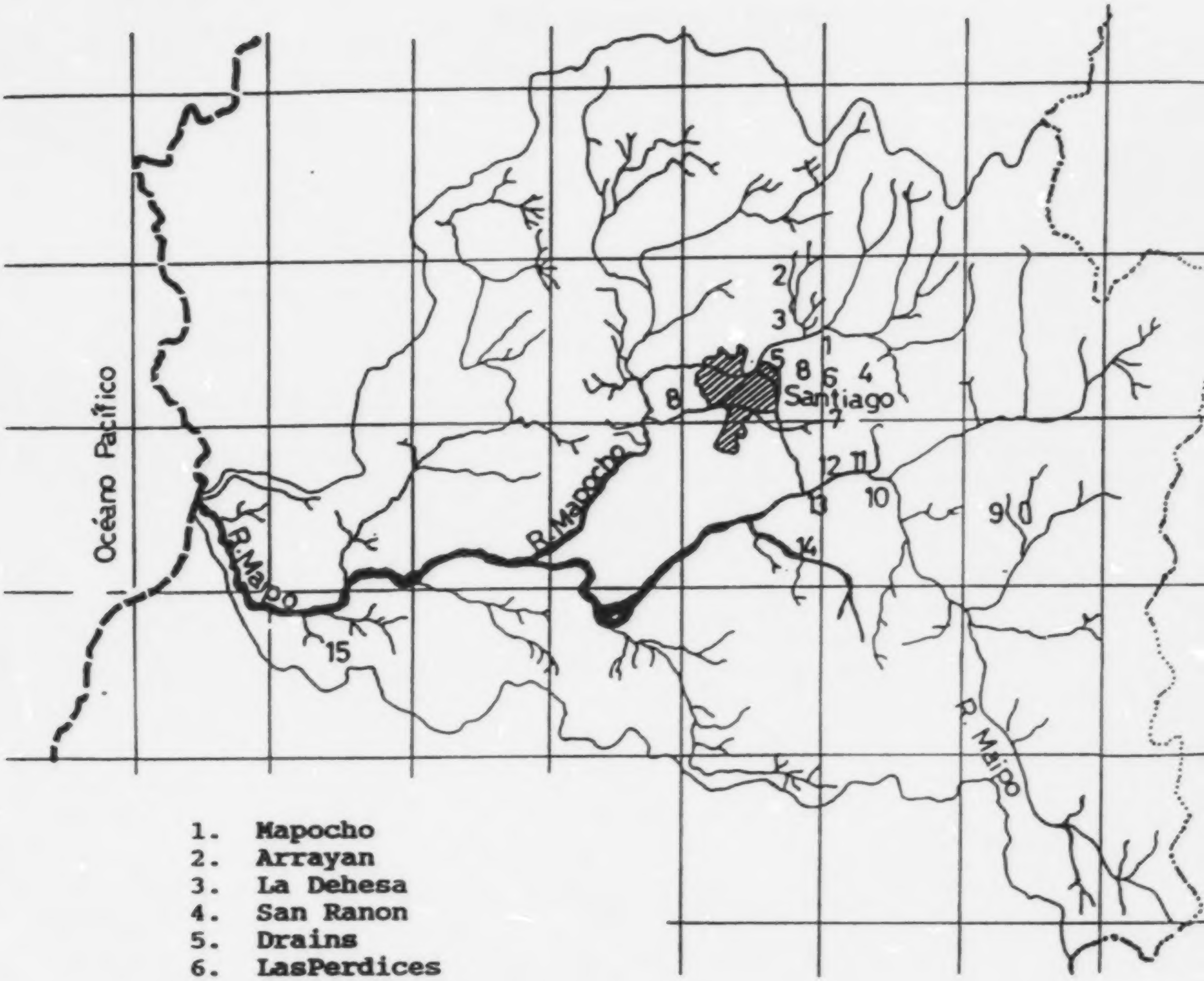
SPEARMAN'S RANK CORRELATION MATRIX FOR THE DIFFERENT WATER QUALITY TESTS IN CHILEAN RAW WATERS*

	Turbidity	Total Coliforms	Faecal Coliforms		
			EC	A-1	MFC
Total Coliforms	.382				
EC	.396	.444			
A-1	.632	.313	.677		
MFC	.766	.336	.588	.826	
Coliphage	.376	.029	.350	.308	.387

* All correlations, except coliphage to total coliforms, positive and significant (p < .01)

FIGURE 1

POTABLE WATER SOURCES OF METROPOLITAN REGION,
SANTIAGO, CHILE



1. **Mapocho**
2. **Arrayan**
3. **La Dehesa**
4. **San Ranon**
5. **Drains**
6. **LasPerdices**
7. **La Turca**
8. **Urban Wells**
9. **Laguna Negra**
10. **Maipo**
11. **El Manzano**
12. **El Canelo**
13. **Vizcachas, Vizcachitas**
14. **El Clarillo**
15. **Rural Wells**

EVALUATION OF THE COLIPHAGE PROCEDURE AND THE PRESENCE/ABSENCE TEST AS SIMPLE RAPID ECONOMICAL METHODS FOR SCREENING POTABLE WATER SOURCES AND POTABLE WATER SUPPLIES IN PERU

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ABSTRACT

Different methods were compared for the detection of total coliforms, faecal coliforms and coliphages in 80 triplicate samples of raw water and 160 triplicate treated water samples. The techniques used for raw water were: the coliphage test, conventional MPN using EC medium versus A-1 broth at 44.5°C and membrane filtration procedures using M-FC Gelman and M-FC Iso-grid membranes. All river samples were positive using the coliphage technique, and the relationship between coliphages/faecal coliforms was 1:10. Different relationships were found in other kinds of water examined.

In 36% of the spring and well water samples with low numbers of faecal coliforms, no coliphages were detected. The A-1 test showed similar degrees of selectivity and specificity as that obtained by EC medium.

The Presence/Absence (P/A), H_2S and coliphage tests were used to test 160 samples of treated water (110 of drinking water from distribution lines and wells and 50 of bottled water). In 42% of the drinking water samples, one or more indicators of microbial contamination were found while 18% were positive only by the P/A test. For faecal coliforms, the P/A test, the H_2S test and the MPN test produced 18.2%, 16.4% and 11.8% positive samples, respectively. Therefore the P/A test was the most sensitive technique.

In treated water, 34% of the samples were positive for coliphage and total faecal coliforms while 18% of the samples were positive for coliphage and negative for total and faecal coliforms.

INTRODUCTION

Peru has three different geographical regions: Coast, Highlands and Tropical Jungle. Due to the fact that Peru's territory is highly irregular, the basic services for sanitation are deficient. A large percentage of the rural and suburban areas lack drinking water and sewage treatment systems. This factor is responsible for the high incidence of infant mortality due to water-borne infectious diseases.

A study conducted by the Ministry of Housing indicates that the high rate of urban population growth is due to a high birth rate and a large migration from rural areas. This results in the creation of communities with poor housing around the perimeter of the big cities which lack all kinds of services. These communities are called "growing villages" (pueblos jovenes).

The water supply and sanitation services have a mixed administration. SENAPA is the national authority for water supply and its responsibility is to coordinate water supply activities in cities outside the capital city. SENAPA controls 53 water treatment plants. Nineteen of them have laboratories with basic equipment for physical, chemical and microbiological analysis.

Lima, the capital city, has its own water authority (SEDAPAL), which is responsible for the surveillance of drinking water quality and sewage facilities. SEDAPAL is responsible for a population of about 7 million people, but only has two laboratories, one in its headquarters and the other in the water treatment plant. According to Peruvian legislation, the Ministry of Health is responsible for protecting the water resources, disinfecting and treating of water, and water sampling. However, the Basic Rural Sanity Direction (DISABAR) is responsible for rural area service and controls natural water resources.

According to Peruvian legislation, the surveillance and quality control of waters for the 20 million inhabitants of Peru (65% located in cities), is the responsibility of the Ministry of Health through its Technical Directorate of the Environment (DITESA), and its Departmental Units in coordination with the regional hospitals.

Until 1984 there did not exist any formal body/committee for the development, evaluation and adoption of standard analytical methods for water quality. Therefore, the few operative laboratories analyzed different parameters, expressed their results in various ways and applied different limits. At this moment the World Health Organization (WHO) guidelines have been adopted and are being implemented throughout the country.

A WHO report dated May 1987 stated: "The existing health service and water authority laboratories in Peru are both poorly structured and equipped; nonetheless, the Ministry of Health does have a basic centralized laboratory service within its Technical Directorate of the Environment (DITESA). It provides a unified Central Reference Laboratory fully equipped for microbiological, inorganic, organic and organoleptic analytical functions. Initially, as a decade objective, it has been recommended that DITESA should also develop regional health laboratories and support surveillance using basic portable field test kits in the provincial towns and cities."

At this moment DITESA is initiating a Water Surveillance Program with the support of the Overseas Development Administration of the government of the United Kingdom through DELAGUA Ltd. (Public Health Consultants), CEPIS/PAHO/WHO - Lima.

For the monitoring of water in the rural area, water testing kits (MILLIPORE and DELAGUA) are used. The DELAGUA portable laboratory kit is capable of performing physical and chemical tests such as: pH, residual chlorine, turbidity and conductivity.

National water quality standards for microbiological water quality are based on the coliform test. However, very few bacteriological analyses of water are carried out in the country. This is due to the lack of qualified technical and administrative personnel as well as the lack of laboratory facilities. Only in the large cities and the capital city (Lima) is this activity performed to any extent.

Recent information indicates that the Drinking Water Quality Control Laboratory of SEDAPAL processes 400 water samples monthly. The microbiological parameters measured include total coliforms and faecal coliforms. The methodology used is the one recommended by "Standard Methods", APHA, 16th edition (1985).

From the above it was obvious that there was a great need in Peru for the development and widespread use of simple, inexpensive microbiological procedures to evaluate Peruvian raw drinking water source and potable water supplies. The CLEIBA laboratory, with the sponsorship of the IDRC (International Development Research Centre, Ottawa, Canada), undertook a study on the feasibility and applicability of a variety of simple, sensitive and inexpensive bacteriological water quality testing procedures (P/A test, H_2S paper strip test and A-1 broth test) in raw and treated potable waters. Included in this study was the evaluation of a simple, inexpensive and quick coliphage enumeration procedure for testing both raw and potable waters.

These procedures were compared to the traditional and Peruvian bacteriological tests. The results of the IDRC-sponsored study are presented below.

MATERIALS AND METHODS

Eighty water samples, collected in triplicate from rivers, springs and wells were tested for:

1. Coliphage concentration, following the revised method in APHA (1985), using the addition of 0.08 mL of 1% 2,3,5 triphenyl tetrazolium-chloride;

2. Faecal coliforms by,

   (a) MPN technique using LST broth 35°C, BGB. 2% 35°C, and EC medium 44.5°C;
   (b) A-1 Broth at 44.5°C (APHA Standard method);
   (c) MF techniques using M-FC agar at 44.5°C and Gelman GN-6 0.45 micron membrane filter; and
   (d) QA square grid MF technique using M-FC agar at 44.5°C using hydrophobic square gridded membrane filters developed by Sharpe (1981) and marketed as Iso-grid Method (QA Laboratories, Toronto, Canada).

Twenty water samples in triplicate were tested to identify the faecal coliforms enumerated by the MPN technique by EC medium and A-1 broth as well as by the MF procedures using Gelman and Iso-grid membranes. IMVIC, oxidase, lysine decarboxylase and ornithine decarboxylase tests were used to identify these faecal coliforms.

Eighty water samples in triplicate were tested to evaluate the sensitivity of the two membrane procedures in their detection of faecal coliforms.

One hundred and ten potable water samples collected from distribution lines and wells which were subjected to chlorination were tested by the P/A test (Clark _et al_, 1962) as detailed in APHA Standard Method, Section 908E. Positive tests were confirmed for total coliforms, faecal coliforms, _Pseudomonas aeruginosa_, _Clostridium perfringens_, _Aeromonas_, faecal streptococci, and _Staphylococcus aureus_.

The above 110 potable water samples were also tested using the hydrogen sulphide paper strip technique (Hazbun and Parker, 1983).

Positive samples were confirmed for coliforms, faecal coliforms, _Salmonella_, _Proteus_ and _Clostridium_.

The above 110 potable water samples were also tested by total and faecal coliforms tests (APHA Standard Methods, 1985) using the 5-tube MPN procedure with lauryl tryptose broth and brilliant green lactose bile broth for total coliform and confirmation in EC broth for faecal coliforms.

Fifty potable water samples were tested for coliphage using 100 mL of water sample, 100 mL of media and petri dishes 150 x 20 mm. Also, 50 bottled drinking water samples were examined, 25 with gas and 25 without gas by the P/A test, H_2S test, total and faecal coliforms and heterotrophic plate count (HPC).

RESULTS AND DISCUSSION

Table I summarizes the results obtained from the 80 samples collected in triplicate from rivers, springs and wells. One of the rivers, the Rimac River, provides water to the Treatment Plant that supplies drinking water to Lima. The other four rivers provide water for agricultural purposes. In the rural areas the water supply is from wells and/or springs of which twenty-three well water and nine spring water samples have been analyzed. The rural wells are not protected from external contamination.

Results obtained by the faecal coliform and coliphage techniques from these 80 samples are summarized in **Table II**. Similar maximum and minimum faecal coliform counts were obtained with the EC and A-1 media. **Table III** illustrates that 89.1% of the isolates from the EC medium are E. coli and 86.8% of the isolates from A-1 medium, both give close values. The selectivity for other coliform bacteria (Klebsiella, Enterobacter, Citrobacter) and non-coliform (Aeromonas and others) is low by both media.

The results also indicate that A-1 medium gives a good recovery for faecal coliform detection, with the advantage of having a shorter analysis time (24 hours) than the EC MPN procedure.

Regarding the results obtained through the membrane filtration procedure, we found that faecal coliform populations estimated by the M-FC-Gelman were only 54% of the faecal coliform population estimated by the MPN-EC method and 57% of the faecal coliform population estimated by the A-1 broth procedure for the waters examined.

These low populations estimates by the membrane filtration procedure are in agreement with the work of Jacobs et al (1986) who found that the membrane filter technique detected 64% of total coliforms versus 82% detected by MPN technique. Our faecal coliform population estimates by the MF technique were even lower than those found by Jacobs et al (1986). We believe the reason for the lower selectivity of the MF technique could be the existence of injured coliform cells that are not counted or that the M-FC selective media inhibits the injured coliform cells.

When the membranes (Gelman and Iso-grid) were evaluated for selectivity with regard to E. coli resuscitation and growth, the M-FC-Gelman procedure detected 92%, the M-FC- Iso-grid, 84%, with respect to the EC and A-1 MPN procedures. With the M-FC Iso-grid procedure, it was found that 22% of the faecal coliform isolates were Klebsiella spp.

The efficacy of the Gelman and Iso-grid membrane filters appear to be equivalent from **Table III** data.

To determine the selectivity of the two membranes for E. coli, 848 faecal coliform isolates from M-FC Gelman and 876 faecal coliform isolates from M-FC- Iso-grid were identified.

In **Table IV** it can be seen that 80.4% of the FC isolates were E. coli using the Gelman filter and M-FC agar and 67.3% of the isolates were E. coli using the Iso-grid membrane and M-FC agar. Klebsiella were found in considerable numbers by both procedures, 9.1% by Gelman membrane and 20.8% by the Iso-grid membrane procedure.

Tobin and Dutka (1977) found significant differences in faecal coliform and coliform recoveries in nine types of membrane filters. These differences were due to many factors: conformation of the membrane pore, liquid flux, presence of heavy metals in the membranes and the type of membrane sterilization.

It is important to note that the Gelman membranes were sterilized by autoclave while the Iso-grid membranes came in individual sterilized containers.

Other observations on the handling of the membranes are:

1. In the faecal coliform test by membrane filtration other colonies than E. coli such as Citrobacter sp. and Klebsiella sp. also grow and develop a blue colour, but less intense than typical E. coli and thus it is always necessary to confirm the presumptive presence of E. coli. This increases the analysis time.

2. With regard to the counting of the colonies on the membranes, we found that the Gelman membrane has a limitation because the number of surface colonies that can be counted ranged from 80 to 100. The Iso-grid membrane has 1,600 cells in which an organism can form a colony. This makes the counting procedure easier and allows a high counting range up to 1,600 CFU/membrane.

Summarizing, the membrane filtration method is quick and easy, but based on the data obtained, the efficiency must be increased and procedures modified to accommodate injured, stressed cells.

In rivers, the coliphage test gives values with a decimal reduction (90% of the population) in comparison to the number of faecal coliforms obtained through the MPN (EC) technique. The relation between coliphages/faecal coliforms is 1:10. In springs, the reduction is of 87% and the relation is 1:7.7 and in wells the reduction is of 72% and the relation is 1:3.6 (**Table I**).

A direct relationship between coliphages and faecal coliforms was observed when the number of faecal coliforms was less than 1,000/100 mL when the concentration of faecal coliforms was higher there was consistent relationship between coliphages and faecal coliforms and the number of coliphages enumerated were non-predictable.

In **Table II** it can be seen that 100% of the river water samples are coliphage positive. In well and spring water the percentages were lower: 47% and 44%, respectively. From 23 well water samples analyzed, nine were negative for coliphage, but positive for faecal coliforms. These coliphage negative samples had low levels of faecal coliforms (0.7-19/100 mL). In the three spring water samples which were negative for coliphages, low levels of faecal coliforms (0.6-400/100 mL) were also observed.

Summarizing, the coliphage test was found to provide similar results to the faecal coliform test in the evaluation of river water. As some coliphage negative results were obtained in spring and well water, we recommend that larger volumes of water samples be tested for coliphages in these waters and that a search be made to find a host strain with a broader spectrum.

Drinking water samples (110) were collected from distribution lines and wells which were subjected to chlorination for examination. The P/A test and H_2S paper strip test were used and compared with the MPN procedure for total and faecal coliforms. The first 60 samples were also tested for heterotrophic counts (HPC) and for the last 50 samples were also tested for coliphage concentrations.

From the 110 drinking water samples, 64 were negative for the P/A, H_2S and total and faecal coliform MPN tests. In **Tables V** and VI, the results obtained in the 46 positive samples (41.8%) for one or more indicators of microbial contamination are given. Only 12 samples (10%) were positive to the P/A test, 20 samples (18.2%) were positive for the P/A and MPN tests and only five samples (4.5%) were positive only by the H_2S test. The heterotrophic plate count varied from 5 to 8.5×10^3 cfu/mL.

Based on P/A and H_2S test results which indicate the potential presence of total coliforms in 27.3% and 16.4% of the positive samples, the total coliform count by the MPN procedure only indicated the presence of total coliforms in only 17.3% of the positive samples. Similarly, the P/A test results indicate the potential presence of faecal coliforms in 18.2% while by the FC MPN technique, only 11.8% of the positive samples were found to contain faecal coliforms.

These results indicate that the P/A test is more sensitive than the MPN test for both total and faecal coliforms detection in these potable waters. Also, the H_2S test gives a recovery percentage of coliforms similar to the one obtained by the MPN test.

Jacobs et al (1986) in a comparative study of techniques for detection of total coliforms in potable water systems also found a higher sensitivity by the P/A test (88%) against 82% for the MPN test and 64% for the membrane filtration procedure.

Of the 50 water samples examined, 17 were positive (34%) within the range 1 to 57 PFU/100 mL with a mean value of 11.6 PFU/100 mL (Table VI). It was possible to detect small number of PFU in large volumes of water (100 mL) because the APHA technique sensitivity was increased using five times the quantity of culture media and larger petri dishes (150 x 20 mm).

It is important to note that 18% of the positive samples showed coliphages as the only faecal indicator. If this percentage (18%) is compared to the one found through the MPN test (11.8%), it can be seen that the efficiency of the coliphage detection test with respect to the conventional technique (TC MPN) is in the order of 142%. El-Abagy et al (1988) also found coliphages present in drinking water samples that were negative for total and faecal coliforms.

Sim and Dutka (1987) stated that drinking water free from coliforms can still contain pathogenic micro-organisms. Many water samples free from coliforms contained varying concentrations of coliphages; this indicates that the water has had an inadequate treatment and thus enteric human virus could also survive this treatment process.

Fifty bottled water samples were analyzed; 25 of them with gas (10 in 450 mL glass bottles, five in siphon glass bottles and 10 in 2-litre plastic bottle dispensers); 25 samples without gas, all in 20 litre plastic bottle dispensers.

In the results from bottled waters containing microbial contaminants (eight with gas and 14 without gas), 32% of the bottled waters with gas had microbial contamination; seven samples (28%) contained Pseudomonas aeruginosa and five (20%) had total and faecal coliforms by the P/A method. No coliforms were detected by the MPN technique in any of the samples assayed. The range of heterotrophic counts varied from 30 to 6.5 x 10^2 cfu/mL with a mean value of 2.5 x 10^3 cfu/mL.

Fifty-six percent of the bottled water samples without gas were positive for one or more indicators of bacterial contamination: 10 samples (40%) contained Pseudomonas aeruginosa, eight samples (32%) total coliforms, two samples (8%) faecal coliforms by the P/A test, five samples (20%) were positive to the H_2S test (isolating Citrobacter freundii, Klebsiella sp., E. coli, Pseudomonas aeruginosa). The heterotrophic plate counts varied from 1.1 x 10^2 to 1.6 x 10^4 cfu/mL with a mean value of 2.3 x 10^3 cfu/mL.

Comparing all the results from the bottled drinking water study, it was found that the P/A test results indicate the presence of TC and FC in 26% and 7% of the samples while the results obtained by the traditional MPN tests indicate the presence of TC and FC in 8% and 2% of the sample. Therefore, we can deduce that the P/A test is more sensitive than the MPN test.

The H_2S technique results were compared to MPN technique in 50 samples of potable bottled water. For the H_2S technique, five samples (10%) were positive and E. coli was identified as the causal agent. For the MPN technique, four samples were positive for TC (8%) and one sample for FC (2%). From these results we can assume that the H_2S technique is more sensitive for potential human health hazards than the MPN technique in the testing of potable water supplies.

CONCLUSIONS

On the basis of all the data generated in this study, the following conclusions were reached:

1. The ratio of coliphage to coliform was 1:7. This is the mean value for all the types of raw water analyzed. In drinking water the coliphage technique showed higher efficiency than the MPN procedures (142%), but was necessary to use five times more the quantity of the culture media and water sample (100 mL) as indicated in the basic technique; this added media made the procedure more expensive.

2. The P/A test is more sensitive for the detection of total coliforms and faecal coliforms in drinking water than the conventional MPN test.

   The H_2S paper strip test showed the same sensitivity as the MPN test for the total coliforms determination, but was less sensitive for faecal coliform determination. The H_2S test is very useful for field work. It was observed that incubation at 35°C gives better results than incubation at 22°C.

3. The MPN procedure for the detection of faecal coliforms using the A-1 broth media gives good sensitivity and specificity after 24 hours of incubation when compared with the EC media. Therefore, A-1 broth media could be used as an alternative to the EC media resulting in a reduction in analysis time and media costs.

4. In the detection of faecal coliforms using the membrane filtration technique, M-FC Gelman and M-FC Iso-grid, it was found that these systems are not very selective because of lower E. coli recovery when compared with the MPN (EC and A-1) technique.

RECOMMENDATIONS

1. Based on the results obtained in the detection of coliphages in raw waters it would be interesting to make a comparative study of coliphages versus coliforms. This study could use the same enumeration system for both indicators, that is the MPN technique, and be evaluated on surface waters (springs) and rural well waters.

2. A study of rural potable water supplies employing the H_2S and P/A tests should be initiated to fill our knowledge gap in this area.

3. Evaluate the 10-tube MPN technique for coliphages in drinking water and also use this procedure in Recommendation #2.

4. Perform a bacterial injury study in faecal coliforms during the membrane filtration procedure.

5. To try and understand the lack of sensitivity of the MF technique in Peruvian waters, it would be interesting to carry out a study to understand whether injured faecal coliforms are not enumerated by the MF procedure or are they injured by the MF procedure and thus not counted.

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TABLE I

INCIDENCE OF FAECAL COLIFORMS IN RAW WATER

PARAMETERS	Rivers		Springs		Wells	
	Mean	Range	Mean	Range	Mean	Range
Total Coliforms[1]	3.4×10^4	$3 \times 10^2 - 7 \times 10^5$	2.9×10^3	$3 - 1.6 \times 10^4$	1.7×10^3	$4 - 1.1 \times 10^4$
Faecal Coliform (EC)[1]	2.0×10^4	$70 - 2.6 \times 10^5$	1.4×10^3	$<2 - 8 \times 10^3$	1.1×10^3	$<2 - 5.7 \times 10^4$
Faecal Coliform (A-1)[1]	1.7×10^4	$90 - 2.4 \times 10^5$	1.9×10^3	$<2 - 9 \times 10^3$	8.4×10^2	$<1 - 2.2 \times 10^4$
Faecal Coliform[2] (MFC Gelman)	1.0×10^4	$63 - 2.4 \times 10^4$	7.3×10^2	$<1 - 4.6 \times 10^3$	6.8×10^2	$<1 - 2.5 \times 10^3$
Faecal Coliform[2] (MFC Iso-grid)	1.1×10^4	$80 - 3.8 \times 10^4$	7.1×10^2	$<1 - 4.4 \times 10^3$	6.2×10^2	$<1 - 2.9 \times 10^3$
Coliphage[3]	2.0×10^3	$5 - 1.5 \times 10^4$	1.9×10^2	$5 - 1.6 \times 10^3$	3.1×10^2	$<5 - 1.4 \times 10^3$
Total samples	48		9		23	

[1] MPN/100 mL

[2] CFU/100 mL

[3] PFU/100 mL

TABLE II

PERCENTAGE OF POSITIVE SAMPLES FOR FAECAL COLIFORMS AND COLIPHAGES

Sources No. of Samples	Faecal Coliforms		Coliphages	
	No. Positive Samples	%	No. Positive Samples	%
River (48)	48	100	48	100
Well (23)	20	86	11	47
Spring (9)	7	77	4	44

TABLE III

PRESENCE OF E. COLI, OTHER COLIFORMS AND NON COLIFORMS IN EC MEDIUM, A-1 BROTH, MFC (GELMAN MEMBRANE) AND MFC (ISO-GRID MEMBRANE) (Expressed in %)

Bacteria Id / Culture Media / Water Source	E. coli				Kiebsiella				Enterobacter				Citrobacter				Aeromonas				Others			
	EC	A-1	MFC		EC	A-1	MFC		EC	A-1	MFC		EC	A-1	MFC		EC	A-1	MFC		EC	A-1	MFC	
			G^1	I^2			G^1	I^2			G^1	I^2			G^1	I^2			G^1	I^2			G^1	I^2
Rivers	94.2	89.3	84.3	88.9	3.0	8.3	4.9	7.1	1.6	1.0	3.5	3.0	1.0	0.3	5.3	-	-	0.3	0.8	0.8	-	0.6	0.4	0.4
Springs	94.8	83.8	88.3	70.4	5.1	14.5	6.9	22.7	-	1.6	2.3	6.8	-	-	2.3	-	-			-				-
Wells	81.9	84.6	75.0	47.3	14.5	14.5	19.2	33.9	3.5	0.4	2.8	14.2	-	0.4	0.9	4.4	-	-	0.9	-	-	-	0.9	
Total	89.1	86.8	82.2	74.6	8.0	11.4	9.1	16.7	2.3	0.6	3.2	6.7	0.4	0.3	3.7	1.3	-	0.3	0.8	0.5	-	0.3	0.8	0.5

[1] Gelman membrane

[2] Iso-grid membrane

TABLE IV

PRESENCE OF E. COLI, AND OTHER ENTEROBACTERIACEAE IN THE TWO MEMBRANE FILTRATION PROCEDURES

(Expressed in %)

Microorganism	M-FC GELMAN		M-FC. ISO-GRID	
	No.	%	No.	%
E. coli	683	80.4	589	67.3
Enterobacter sp.	28	3.2	45	5.1
Klebsiella sp.	78	9.1	178	20.3
Citrobacter sp.	40	4.7	44	5.0
Aeromonas sp.	10	1.1	10	1.1
Lact (-)	1	0.1	4	0.4
Not identified	8	0.9	6	0.6
Total	848		876	

TABLE V

POTABLE WATER SAMPLES, POSITIVE BY ONE OR MORE BACTERIAL INDICATOR TESTS

Water Source	Free Residual Chlorine mg/L	P/A Test/100 mL							H₂S Test/100 mL				MPN/ 100/mL		HPC[8] /mL
		TC[1]	FC[2]	FS[3]	Cl.p[4]	P.a[5]	S.a[6]	Aer[7]	+ or neg. 22°C	+ or neg. 35°C	Bacteria Identified 22°C	Bacteria Identified 35°C	TC	FC	
Distribution system	0.10	P	P	A	A	A	A	A					2	<2	210
Distribution system	0.25	P	P	A	A	A	A	P	+	+	Aeromonas	Citrobacter P.aeruginosa Aeromonas			200
Distribution system	0.0	P	P	A	A	P	A	A	-	-			<2	<2	298
Distribution system	0.0	P	P	A	A	P	A	A	-	-			<2	<2	195
Distribution system	0.1	A	A	A	A	P	A	A	-	-			<2	<2	6200
Distribution system	0.1	A	A	A	A	P	A	A	-	-			<2	<2	7200
Well	0.0	P	P	A	A	A	A	A	-	-			130	<2	3500
Distribution system	0.0	P	A	A	A	P	A	A	-	-			<2	<2	298
Well	0.0	P	A	A	A	A	A	A	-	-			130	<2	3500
Distribution system	0.0	P	A	A	A	P	A	A	-	-			<2	<2	298
Well	0.0	P	P	A	A	A	A	A	-	-			>1600	1600	8500
Well	0.1	P	A	A	A	A	A	A	-	-			<2	<2	11
Distribution system	0.25	P	A	A	A	P	A	A	-	-			<2	<2	13
Distribution system	0.25	A	A	A	A	A	A	A	+	+	Citrobacter	Citrobacter Clostridium	<2	<2	11

TC[1] - total coliforms

FC[2] - faecal coliforms

FS[3] - faecal streptococci

Cl.p[4] - Clostridium perfringens

P.a[5] - Pseudomonas aeruginosa

S.a[6] - Staphylococcus aureus

Aer[7] - Aeromonas

HPC[8] - heterotrophic plate count

TABLE VI

RESULTS OF BACTERIAL AND COLIPHAGE TESTS ON POTABLE WATER SAMPLES COLLECTED FROM DISTRIBUTION LINES

Water Source	Free Residual Chlorine mg/L	P/A Test/100 mL							H_2S Test				TC	FC	Coliphage PFU[8] /100 mL
		TC[1]	FC[2]	FS[3]	C.p[4]	P.a[5]	S.a[6]	Aero[7]	+ or neg.		Bacteria Identified		MPN /100 mL		
									22°C	35°C	22°C	35°C			
Well	0.0	P	P	A	A	A	A	A	+	+	Citrobacter Not confirmed	Citrobacter E. coli	<2	<2	<1
Well	0.0	P	P	A	A	A	A	A	+	+	Citrobacter Not confirmed	Citrobacter E. coli	4	4	1
Well	0.0	P	P	A	A	A	A	A	-	+		Citrobacter E. coli	12	9	<1
Well	0.0	P	P	A	A	P	A	A	+	+	Citrobacter Not confirmed	Citrobacter E. coli	2	2	<1
Well	0.0	P	P	A	A	A	A	A	-	+		Citrobacter E. coli	21	13	13
Well	0.0	A	A	A	A	A	A	A	+	+	Not confirmed	Citrobacter E. coli	<2	<2	8
Well	0.0	P	P	A	A	A	A	A	-	+	Citrobacter	Citrobacter E. coli	4	<2	15
Well	0.0	A	A	A	A	A	A	A	+	+	Not confirmed	Citrobacter E. coli	<2	<2	9
Well	0.0	P	P	A	A	A	A	A	+	+	Not confirmed	Citrobacter E. coli	2	2	5
Well	0.0	A	A	A	A	A	A	A	-	-			<2	<2	2
Distribution system	0.0	A	A	A	A	A	A	A	-	-			<2	<2	1
Distribution system	0.0	A	A	A	A	A	A	A	-	-			<2	<2	12
Distribution system	0.1	A	A	A	A	A	A	A	-	-			<2	<2	3
Distribution system	0.1	A	A	A	A	A	A	A	-	-			<2	<2	57
Distribution system	0.1	A	A	A	A	A	A	A	-	-			<2	<2	1
Distribution system	0.0	A	A	A	A	A	A	A	-	-			<2	<2	4
Distribution system	0.0	A	A	A	A	A	A	A	-	-			<2	<2	14
Well	0.0	P	P	A	A	A	A	A	+	+	Citrobacter E. coli	Citrobacter E. coli	21	21	9
Well	0.0	P	P	A	A	A	A	A	+	+	Citrobacter E. coli	Citrobacter E. coli	26	6	2
Distribution system	0.5	A	A	A	A	A	A	A	-	-			2	<2	<1
Well	0.0	P	A	A	A	A	A	A	+	+	Citrobacter	Citrobacter	26	4	<1
Well	0.0	P	P	A	A	A	A	A	-	-			2	<2	<1
Well	0.0	P	P	A	A	A	A	A	+	+	Citrobacter	Citrobacter	130	11	<1
Distribution system	0.0	P	A	A	A	A	A	A	-	-			<2	<2	<1
Distribution system	0.0	P	A	A	A	P	A	A	-	-			2	<2	<1
Well	0.0	A	A	A	A	A	A	A	+	-	Pseudomonas		<2	<2	<1
Well	0.0	A	A	A	A	A	A	A	+	-	Pseudomonas		<2	<2	<1
Well	0.0	P	P	P	A	A	A	A	+	+	Citrobacter	Citrobacter	26	9	21
Well	0.0	P	P	A	A	A	A	A	-	-			7	4	<1
Distribution system	0.25	P	A	A	A	A	A	A	-	-			<2	<2	<1
Distribution system	0.1	P	A	A	A	A	A	A	-	-			2	<2	<1
Well	0.0	P	P	A	A	A	A	A	-	-			30	8	<1

TC[1] - total coliforms
FC[2] - faecal coliforms
FS[3] - faecal streptococci

C.p[4] - Clostridium perfringens
P.a[5] - Pseudomonas aeruginosa
S.a[6] - Staphylococcus aureus

Aero[7] - Aeromonas spp.
PFU[8] - plaque forming units

EVALUATION OF COLIPHAGE TESTS AND OTHER SIMPLE MICROBIOLOGICAL METHODS FOR THE EXAMINATION OF DRINKING WATER AND CLASSIFICATION OF WATER SOURCES

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ABSTRACT

Raw and treated water samples from S. Paulo, Brazil, were examined by conventional MPN and MF tests. The results of these tests were compared with those obtained by one of the following procedures: the one-step MPN test for faecal coliforms using A-1 medium, the coliphage test, the P/A test and the H_2S paper strip test.

Results are presented and assessed for sensitivity, specificity, correlation, economics and ease of application of each method.

INTRODUCTION

The bacteriological control of drinking water is of utmost importance from two aspects: quality and quantity, both of which are important from the point of view of human health.

In Brazil, the control of drinking water quality is under the jurisdiction of the Ministry of Health and Secretaries of Health, but in most of the States this activity has been delegated to the Agencies responsible for Environmental Control. The legislation for drinking water quality is based on methods detailed in APHA (1985), specifically total coliform populations estimated by MPN or MF techniques, the number of samples and frequency of sampling (Brazil, 1977).

However, not all of the States are able to meet these regulations due to the lack of funds and/or qualified personnel. Approximately two-thirds of the Brazilian States have potable water quality control and this is performed only in the Capitals and main cities.

The smallest cities have neither bacteriological laboratories nor adequate number of well-trained personnel for operating water treatment plants. For instance, a study completed by CETESB[a] in the State of S. Paulo, found that out of 280 cities (corresponding to 7 million inhabitants), which had water treatment plants, 45% had poor conditions of operation and out of these, 38.9% (109 cities) produced water considered non-potable according to Brazilian legislation (CETESB, personal communication). Some basic sanitary information about Brazil for 1983 is presented in **Table I.**

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[a] CETESB - Companhia de Tecnologia de Saneamento Ambiental,
S. Paulo - SP., Brazil 01329.

Brazil has 26 companies responsible for Water and Sanitation, with a staff of 80,000 (Moitta, 1984), but the number of people involved with bacteriological control of water is approximately 180 (Sanchez, P.,[b] personal communication).

Information concerning sanitation in rural areas is variable, for instance data presented by ABES[c] (1987) are different from that reported by Moitta (1984). ABES reported that the 1980 demographic census revealed that the rural population in Brazil was 38 million, corresponding to 32% of total population. The distribution of this population was not uniform varying from 8% of population in the State of Rio de Janeiro to 69% in the State of Maranhao.

Only 3% of the rural inhabitants had public water supplies, 34% received water of varying quality originating from public water sources, semi-protected reservoirs, etcetera, and 63% have water in their residence or nearby, but of poor quality.

The problems with excreta disposal was more critical, as only 1% of the rural population was connected to a sewer network, 5% had septic tanks, 31% disposed their excreta in rudimentary tanks and 62% had no organized excreta disposal system. The diseases related to poor sanitation conditions such as hepatitis, typhoid fever, and parasite infections are the main ones found in the small rural Brazilian communities. Because of this obvious problem, a National Program for Rural Sanitation was established and has as its goals for the 1986-89 period, the installation of 45,000 water supply systems to serve 337,000 houses in 40,000 small communities with populations of up to 5,000 inhabitants.

In relation to surface water quality, as this is controlled and monitored by the States with no centralized information source, it is difficult to get a comprehensive picture of this water quality. For example, in the State of S. Paulo of the 8,844 km of rivers studied from eight hydrographic zones, 73.5% had good quality water, 18% regular and 8.5% poor water quality (CETESB, 1984).

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[b] Sanchez P.S. - Nominata e Estatuto do Clama, S. Paulo,
1987, Av. Prof. Frederico Hermann Jr. 345,
S. Paulo - Sp., Brazil, CEP. 01329.

[c] ABES - Projecto Nacional de Saneamento Rural.

Due to the obvious need in Brazil for simple, economical, and reliable methodology to evaluate potable and raw water quality, this study was initiated to respond to those needs. In this study, four tests were evaluated and compared to traditional standard bacteriological water quality methods. Those tests were the P/A (Presence/Absence), H_2S paper strip, A-1 broth (faecal coliforms) and the coliphage enumeration procedure.

MATERIAL AND METHODS

In this study, 297 samples of raw waters that supply water treatment plants in the Greater S. Paulo area and 100 samples of drinking water were analyzed.

The following tests were applied: standard faecal coliform (EC) MPN test, faecal coliform membrane filter procedure (MFC), with the modification for stressed organisms, faecal coliform MPN using A-1 medium (APHA, 1985) and the coliphage test (APHA, 1985). In 25% of samples, Presence/Absence (P/A) test (Clark, 1969) and the H_2S paper strip test (Manja, et al, 1982) were also performed.

In order to evaluate the specificity of faecal coliform methods, cultures isolated were submitted to identification (Edwards and Ewing, 1972).

In drinking water the tests performed were MF total coliforms with M Endo LES agar (MF-TC) (APHA, 1985), P/A test, H_2S paper strip test and coliphages.

RESULTS AND DISCUSSION

Table II presents the median values for each indicator or technique and for various raw water sampling areas. Source 3 is the most polluted one and sources 2 and 4 have good quality water. These data were corroborated by field data and all the indicators, including coliphages, showed the same information.

In **Table III** it can be observed that both media, A-1 and EC, produced higher mean faecal coliform densities than that obtained by the membrane filtration procedure.

Table IV presents the correlations between the indicators and methods. A good correlation was obtained between FC (EC) and FC (A-1); coliphage also presented a good correlation with all the bacteriological methods for FC detection when the overall results were analyzed. However, when individual sources were considered the results varied; for instance, for sources 1 and 2 almost all the correlations among coliphage and FC were not significant at

$p < 0.05$, but for sources 3 and 4 these values were significant ($p < 0.001$ and ranged from 0.54 to 0.76.

Table V gives the percentage of E. coli isolated by each procedure. The results indicate that this bacterium was most frequently recovered by the A-1 medium. These data show that the A-1 procedure has good sensitivity and specificity, with the advantage of shortening the time required for performing the test. This procedure was also less costly (**Table VIII**) than conventional methods.

APHA (1985) Standard Methods recommends the A-1 MPN procedure only for the examination of seawater and treated waste water, but studies indicate that it can also be applied to all natural waters for faecal coliform detection and enumeration as a single step method not requiring confirmation.

Data obtained in 75 raw water samples and 100 drinking water samples were compared to evaluate the percentage of agreement (results positive/positive, negative/negative) between each test or indicator and the percentage of discrepancy. Results are presented in **Tables VI** and **VII**. In raw waters (**Table VI**) one can observe that A-1 MPN test and P/A test for total coliforms (TC) were very sensitive in detecting their specific populations. Coliphages agreed with the other tests at 70% levels, but with the exception of P/A-FC the other tests were more sensitive in enumerating their target populations. The H_2S paper strip test was more sensitive at 30°C and 36 hours of incubation with percent of agreement varying from 66.7 with P/A FC to approximately 90% with other FC tests; for the other tests, when the results showed disagreement, the H_2S paper strip test showed more positive results.

Drinking water results are presented in **Table VII**. A very good agreement was obtained among all the tests. P/A-TC was the test with the most positive results. The detection of other bacteria such as P. aeruginosa, Clostridium perfringens, faecal streptococci, Aeromonas sp., in the absence of positivity in the other tests varied from 8% for MF-TC to 15% for coliphages.

The H_2S test showed also good agreement with the other tests in these waters and also the incubation at 30°C/36 hours presented better results than 22°C incubation.

Table VIII displays the cost and the time factors for the various tests. When labour and materials were considered H_2S test and P/A tests proved to be the most economical tests. The A-1 and coliphage tests were also less costly compared to conventional MF and MPN tests and also produced results in less time.

CONCLUSIONS

Based on this study, one may conclude that the MPN one-step test for faecal coliforms using A-1 medium can be used for the examination of any natural waters when FC standards are concerned. P/A test can be used, with good results, for drinking water evaluation, but in some countries the legislation for drinking waters must be modified to adopt this test as an alternative. H_2S paper strip test can be a good tool as a field test to evaluate bacteriological drinking water quality in rural areas and in localities devoid of laboratory support.

Considering coliphage, this test should be evaluated further to study :

(a) the interferences of physico-chemical characteristics of water;

(b) the possibility of retention by soil materials;

(c) sensitivity to chlorine;

(d) sensitivity of host strains; and

(e) volume of sample tested.

Erratic results were obtained with the coliphage test. For example, in some instances coliphages were isolated from treated waters which did not contain coliforms, and in some wells with high coliform levels no coliphages were recovered. Part of this problem may be due to volume size, for example, coliphage test 20 mL, other tests varying from 55.5 mL to 100 mL of sample. One of the main advantages of the coliphage test is that answers, if positive, can be obtained within four to six hours of sample testing. With a better understanding of the various factors which could influence coliphage population estimation, the potential for this test is unlimited.

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TABLE I

BASIC SANITARY INFORMATION IN BRAZIL (1983)

Population (million)		With drinking water		With sanitary excreta disposal	
		N°	%	N°	%
Total	132.3	87.0	65.7	56.3	42.5
Urban	93.7 (71%)	73.8	78.7	47.9	51.1
Rural	38.6 (29%)	13.2	34.2	8.4	21.8

Adapted from Moitta, F., 1984.

TABLE II

MEDIAN VALUES OBTAINED FOR EACH INDICATOR OR TECHNIQUE AND SOURCE

INDICATORS	TC	FC (EC)	FC (A-1)	FC (MFC)	COLIPHAGE
SOURCE	MPN/100 mL			CFU/100 mL	PFU/100 mL
1	500	80	50	45	10
2	220	4	2	9	<5
3	16,000	3,000	3,000	1,862	680
4	140	8	13	12	<5
5	700	80	70	72	<5
6	700	33	50	46	15

TABLE III

BASIC STATISTICAL DATA FOR OVERALL RESULTS

	TC	FC (EC)	FC (A-1)	FC (MFC)	COLIPHAGE
Mean	6,946	1,448	1,483	628	174
Minimum	<2	<2	<1	<1	<5
Maximum	160,000	50,000	21,000	21,000	4,095
Median	500	50	44	42	5

TABLE IV

CORRELATION MATRIX

	TC	FC (EC)	FC (A-1)	FC (MFC)
FC (EC)	0.76			
FC (A-1)	0.75	0.90		
FC (MFC)	0.70	0.86	0.82	
Coliphage	0.66	0.74	0.71	0.68

$p < 0.001$

TABLE V

EVALUATION OF SPECIFICITY OF CULTURE MEDIA AND TECHNIQUE IN THE DETECTION OF *E. COLI*

CULTURE MEDIA AND TECHNIQUE	EC	A-1	MFC
% *E. coli*	71.9	77.1	68.1

TABLE VI

RAW WATERS, % OF AGREEMENT OR DISAGREEMENT AMONG TESTS (QUALITATIVE RESULTS)

TESTS	FC (EC)			FC (A-1)			M-FC			P/A TC			P/A FC			COLIP.			H_2S_{22}			H_2S_{30}		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
MPN faecal coliforms A-1 (FC A-1)	89.3	6.6	4.1																					
Faecal coliforms M-FC (M-FC)	96.0	0	4.0	93.3	0	6.7																		
P/A total coliforms (P/A TC)	94.7	0	5.3	88.0	8.0	4.0	88.0	12.0	0															
P/A faecal coliforms (P/A FC)	65.3	32.0	2.7	72.0	26.7	1.3	70.7	29.3	0	76.7	23.3	0												
Coliphages (colip.)	74.3	25.7	0	75.7	24.3	0	70.3	29.7	0	72.6	23.3	4.1	74.7	12.0	13.3									
H_2S paper strip test 22°C (H_2S_{22})	70.8	23.6	5.6	77.3	20.0	2.7	77.8	22.2	0	76.8	18.8	4.4	60.9	15.9	23.2	69.5	8.3	22.2						
H_2S paper strip test 30°C (H_2S_{30})	88.9	8.3	2.8	88.9	6.9	4.1	90.3	9.7	0	82.4	8.8	8.8	66.7	5.8	27.5	68.1	4.3	27.6	76.8	1.4	21.8			
H_2S paper strip test 35°C (H_2S_{35})	70.1	26.9	3.0	73.6	20.8	5.5	73.6	26.4	0	78.6	20.0	1.4	75.4	10.1	14.5	69.5	11.1	19.4	62.9	17.1	20.0	69.6	24.6	5.8

A ++, --
B +-
C -+
FC (EC) Conventional MPN faecal coliforms

TABLE VII

DRINKING WATER, % OF AGREEMENT OR DISAGREEMENT AMONG TESTS

	P/A TC			P/A FC			P/A (others)			TC MF			H_2S_{22}			H_2S_{30}			H_2S_{35}		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
P/A FC	96.0	4.0	0																		
P/A (others)	90.0	1.0	9.0	90.0	0	10.0															
TC MF	98.0	2.0	0	96.0	0	4.0	88.0	8.0	4.0												
H_2S_{22}	95.0	4.0	1.0	99.0	0	1.0	91.0	9.0	0	95.0	4.0	1.0									
H_2S_{30}	94.0	3.0	3.0	96.0	0	4.0	90.0	8.0	2.0	94.0	3.0	3.0	97.0	3.0							
H_2S_{35}	92.0	8.0	0	94.0	5.0	1.0	86.0	14.0	0	92.0	8.0	0	93.0	6.0	1.0	90.0	9.0	1.0			
Coliphage	91.0	9.0	0	95.0	5.0	0	85.0	15.0	0	91.0	9.0	0	94.0	6.0	0	91.0	9.0	0	99.0	1.0	0

A = Agreement
B = +-
C = -+

TC MF = Total coliforms membrane filter
P/A (others) = Other indicators detected through P/A test

TABLE VIII

EVALUATION OF THE DIFFERENT INDICATOR TESTS CONSIDERING ECONOMY AND ADVANTAGES

Methods	Cost (labour plus materials) U$	Time of tests performance (minutes)	Time for answer (hours)	COMMENTS
FC (A-1)	9.50	11	24	Better sensitivity and specificity than the other two FC tests assayed.
FC (EC)	18.20	25	72	96 hours for TC.
FC (MFC)	12.50	8	24	Initial investment for equipment such as filter holders and vacuum pumps is high.
P/A	6.10	6	96	5 days or more if other indicators are concerned.
H_2S paper strip	3.50	5	36	Can be used in field conditions.
Coliphage	9.50	18	8	More studies are needed.

EVALUATION OF COLIPHAGE AND PRESENCE/ABSENCE TESTS FOR SCREENING POTABLE WATER SOURCES AND WATER SUPPLIES IN EGYPT

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ABSTRACT

Raw River Nile water and different types of potable water samples including tap water, well water, storage tanks and bottled drinking water were subjected to monthly coliphage counts as well as coliform enumeration (in triplicate) for 14 months. Coliphage counts were carried out by using the tentative APHA technique and the Kott-MPN technique. The results showed that the APHA coliphage estimation procedure was more sensitive than the Kott-MPN technique. A considerable number of potable water samples positive for coliphages were found to be negative for coliforms.

The Presence/Absence (P/A) test showed a higher sensitivity than the coliform procedures for indicator bacteria. Total coliforms were detected in 40 samples of drinking water by the MPN technique whereas 77 samples were positive by the P/A test. All P/A positive samples were confirmed for coliform presence.

INTRODUCTION

River Nile and its branches are the main source of drinking water in Egypt. Raw water from the river is treated by coagulation, filtration and chlorination.

The Egyptian drinking water standards are based on the coliform test. The inadequacy of coliforms as an indicator of pollution has been recorded (Dutka, 1973). The coliphage test is one of the newer procedures which has been receiving attention for testing potable water for faecal pollution (Hilton and Statzky, 1973; Kott et al, 1978; and Stetler, 1984). In addition, the Presence/Absence (P/A) test, a simple, inexpensive drinking water testing procedure, has also been recommended for use in routine laboratories as well as rural and field laboratories to test potable waters.

The goals of this study were to evaluate both the coliphage and P/A tests in a variety of potable water samples collected from

River Nile, Ismailia Canal, distribution lines, storage tanks, wells and bottled waters.

MATERIALS AND METHODS

Samples were collected at monthly intervals in triplicate. **Table I** shows the description of sampling sites at Greater Cairo. The samples were collected from the following sites: raw Nile water (3 sites), Ismailia Canal water (1 site), tap water (3 sites), storage tanks (6 sites), well water (4 sites), and bottled drinking water (3 brand types).

Microbiological Procedures:

1. **Coliphage enumeration:** Two techniques were used, APHA (1985) procedure No. 919C and the Kott-MPN technique (1966). E. coli C (ATCC No. 13706) and local isolates of E. coli (++-- IMVIC type) were used as hosts in both procedures.

2. **Enumeration of coliforms:** Two coliform MPN and two faecal coliform MPN procedures were used for all water samples. APHA (1985) procedure for total coliforms using lauryl tryptose sulphate broth (as presumptive) and brilliant green lactose bile broth (to confirm). The other MPN method for total coliform was using MacConkey broth (as presumptive) with confirmation by subculturing to Levine EMB agar. For faecal coliforms, EC broth tubes were used (MPN), and the A-1 broth MPN procedure as detailed in APHA (1985). For drinking water samples, a 10-tube MPN procedure was used.

3. **Isolation and identification of the faecal coliform isolates:** All positive tubes in the final dilutions of the EC and A-1 broth tubes were subcultured onto MacConkey agar plates and up to 10 different lactose fermenters per plate were fully identified by oxidase test and IMVIC reactions. From all positive Levine EMB agar plates of the final dilution series (MacConkey broth), up to 10 different colony types from each plate were identified.

4. **Presence/Absence (P/A) test:** This procedure was carried out as described in APHA (1985) Section 908E.

RESULTS AND DISCUSSION

Trials for E. Coli Phage Sensitive Isolates

Local E. coli (++-- IMVIC type) isolates were purified and subjected to phage assay through 4 samples of treated water, raw Nile water, influent and effluent sewage samples. The results showed that 3 out of 150 E. coli used as hosts were sensitive to phages. These isolates were compared quantitatively to ATCC 13706 to select the most sensitive E. coli host (Table I). The most efficient hosts for phage assay were found to be local E. coli strain No. 11 and the ATCC strain. Accordingly, these two E. coli were used as hosts for the detection of coliphage in the examined samples.

Correlation Between Coliphage Procedures

Two techniques were carried out for enumeration of coliphage, the APHA procedure and Kott-MPN method. **Figure 1** represents the correlation between these two methods in raw water samples. It can be observed that there was a similarity in response between the ATCC host and local E. coli strains (**Figure 1A**). Here it can be seen visually and statistically ($b = 0.742$; $r^2 = 0.593$) that the APHA coliphage technique works equally well with either E. coli host.

It is clear from **Figure 1B** that there is a propensity for the coordinates to be randomly scattered over the whole field. Moreover, the inclination of regression line is downward. The low value of co-efficient of determination ($r^2 = 0.009$) indicates that the Kott-MPN technique is not as sensitive a method as the APHA coliphage count procedure. Thus, there was no relationship between the APHA and Kott procedures even when different hosts were used (**Figures 1C and 1D**).

In drinking water samples, the greater superiority in sensitivity of the APHA coliphage procedure over the Kott-MPN procedure can be observed in **Figure 2** and **Table II**. **Figure 2** shows that some coordinates lie on the X-axis, indicating that the Kott-MPN coliphage procedure is less sensitive than the APHA coliphage procedure. As shown in Table II, the greater sensitivity of the APHA coliphage procedure over the Kott-MPN technique was recorded. Out of 201 APHA coliphage positive samples, only 25 samples were Kott-MPN positives.

Correlation Among Bacteriological Indicators

Figures 3A and **3B** illustrate the association between coliphage counts (APHA procedure) and total coliforms procedures in raw water samples. The figures show a positive association between coliforms and coliphage.

Table III presents all the samples positive for coliphage, total coliforms and faecal coliforms. Out of 645 samples, 161 were positive only by the coliphage test and only 40 samples were positive for both total coliforms and coliphage. No samples were positive for total coliforms alone. Eight of the 40 total coliform positive samples were also positive for faecal coliforms. This study found, similar to the findings by Sim and Dutka (1987), that many of the coliform free potable water samples contained coliphage, an indication of inadequate treatment.

Figure 4 shows a positive and strong association between EC and A-1 faecal coliform procedures (**Figure 4A**). **Figure 4B** shows a strong association between total coliform APHA and EMB procedures.

Correlation Between P/A Test and Coliform Procedure

Out of the 645 potable water samples tested for contaminants by the P/A test, 77 were found to be positive by the P/A procedure and 40 by MPN procedures (Table IV). These results indicate that the P/A test was much more sensitive than the MPN procedures in detecting polluted potable waters. Similar results were noted by Jacobes et al (1986).

The P/A positive samples were tested for the presence of total coliforms, faecal coliforms, faecal streptococci, Aeronomas spp., Pseudomonas spp., and Clostridium species (APHA, 1985). All the positive P/A samples were found to contain coliforms and 59% of these samples also contained faecal coliforms. Twenty-three of the positive P/A samples also contained faecal streptococci while 33 samples each contained Pseudomonas spp. and Clostridium spp.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the APHA coliphage procedure using E. coli host (ATCC 13706) was found to be the most sensitive procedure to detect polluted potable water. This procedure was also found to be very simple and inexpensive to carry out in the laboratory. The P/A test was found to be the most sensitive, simple and inexpensive bacteriological test for screening potable waters for contaminants.

We recommend without reservation the combination of coliphage and P/A tests for monitoring potable water supplies (tap, bottled, well and storage tanks). Also, due to their extreme simplicity, we strongly recommend that the P/A and coliphage tests be promoted for routine use in rural areas where there is rare and infrequent potable water testing.

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TABLE I

QUANTITATIVE COMPARISONS BETWEEN COLIPHAGE HOSTS

Time	*E. Coli* isolate	PFU/100 mL of			
		Drinking water	River Nile water	Sewage influent	Sewage effluent (ABC's)
1st week	11	12	120	1440	1200
	ATCC	15	130	1480	1210
	80	0	10	350	170
	51	0	20	220	70
2nd week	11	30	210	1535	1135
	ATCC	35	195	1670	1095
	80	10	15	210	115
	51	10	20	190	0
3rd week	11	25	320	1305	1215
	ATCC	20	305	1600	1325
	80	0	20	705	645
	51	0	10	635	405
4th week	11	45	265	1290	1200
	ATCC	105	395	1785	1505
	80	0	105	990	340
	51	5	15	805	355

TABLE II

CENTRE BETWEEN APHA AND KOTT-MPN COLIPHAGE PROCEDURES IN DRINKING WATER

Source	APHA coliphage counts		Kott-MPN coliphage count	
	No. of positives	Range of Means	No. of positives	Range of Means
Tap water	28	3-800	1	2
Storage Tanks	52	3-870	2	1-2
Wells	89	3-2200	18	2-20
Bottled Water	32	3-100	4	3-8
Total	201		25	

TABLE III

SUMMARY OF WATER SAMPLES POSITIVE FOR COLIPHAGE, TOTAL COLIFORMS AND FAECAL COLIFORMS

	Type of Drinking Water				
TEST	Storage Tanks	Tap Water	Well Water	Bottled Drinking Water	Total
Coliphage	52	28	89	32	201
% positive					31.2%
Total coliform	12	0	15	13	40
% positive					6.2%
Faecal	0	0	1	7	8
% positive					1.2%
Total Samples Tested	270	135	132	108	645

TABLE IV

CORRELATION BETWEEN P/A POSITIVE SAMPLES AND COLIFORM POSITIVE IN DIFFERENT TYPES OF DRINKING WATER

	Type of Drinking Water and Number Positive				
TEST	Storage Tanks	Tap Water	Well Water	Bottled Drinking Water	Total
P/A	14	2	40	21	77
Total coliform	12	0	15	13	40
Faecal coliform	0	0	1	7	8
Total Samples Tested	132	135	270	108	

TABLE V

CORRELATION BETWEEN P/A POSITIVE SAMPLES AND COLIFORM POSITIVE IN DIFFERENT TYPES OF DRINKING WATER

	Type of Drinking Water and Number of Routine Tests				
TEST	Storage Tanks	Tap Water	Well Water	Bottled Drinking Water	Total
P/A	14	2	40	21	77
Total coliform	12	0	15	13	40
Faecal coliform	0	0	1	7	8
			0.37%	6.48%	
Total Samples Tested	132	135	270	108	

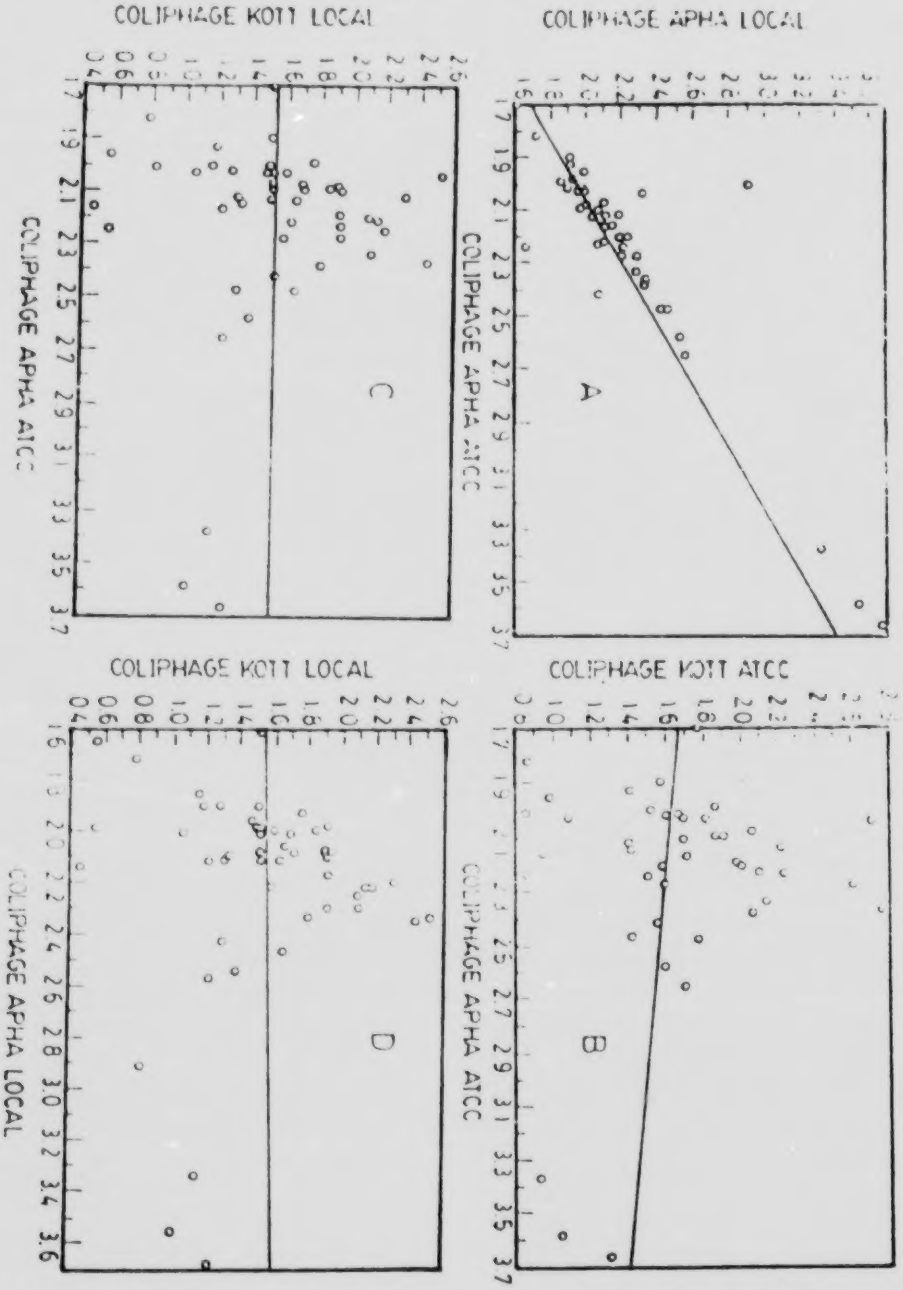
TABLE VI

IDENTIFICATION OF POSITIVE P/A SAMPLES

Designation	**Total Coliforms**	**Faecal Coliforms**	**Faecal Streptococci**	***Aeromonas* *spp*.**	***Pseudomonas* *spp*.**	***Clostridium* *spp*.**
Positive	77	46	23	0	33	33
%	100%	59.74%	29.87%	0	42.86%	42.86%

FIGURE 1

CORRELATION BETWEEN APHA AND KOTT COLIPHAGE PROCEDURES USING LOCAL AND ATCC HOSTS IN RAW WATER SAMPLES



A APHA-Coliphage Counts (Local-ATCC)
$Y = 0.742 x + 0.534$ ($r^2 = 0.593$)

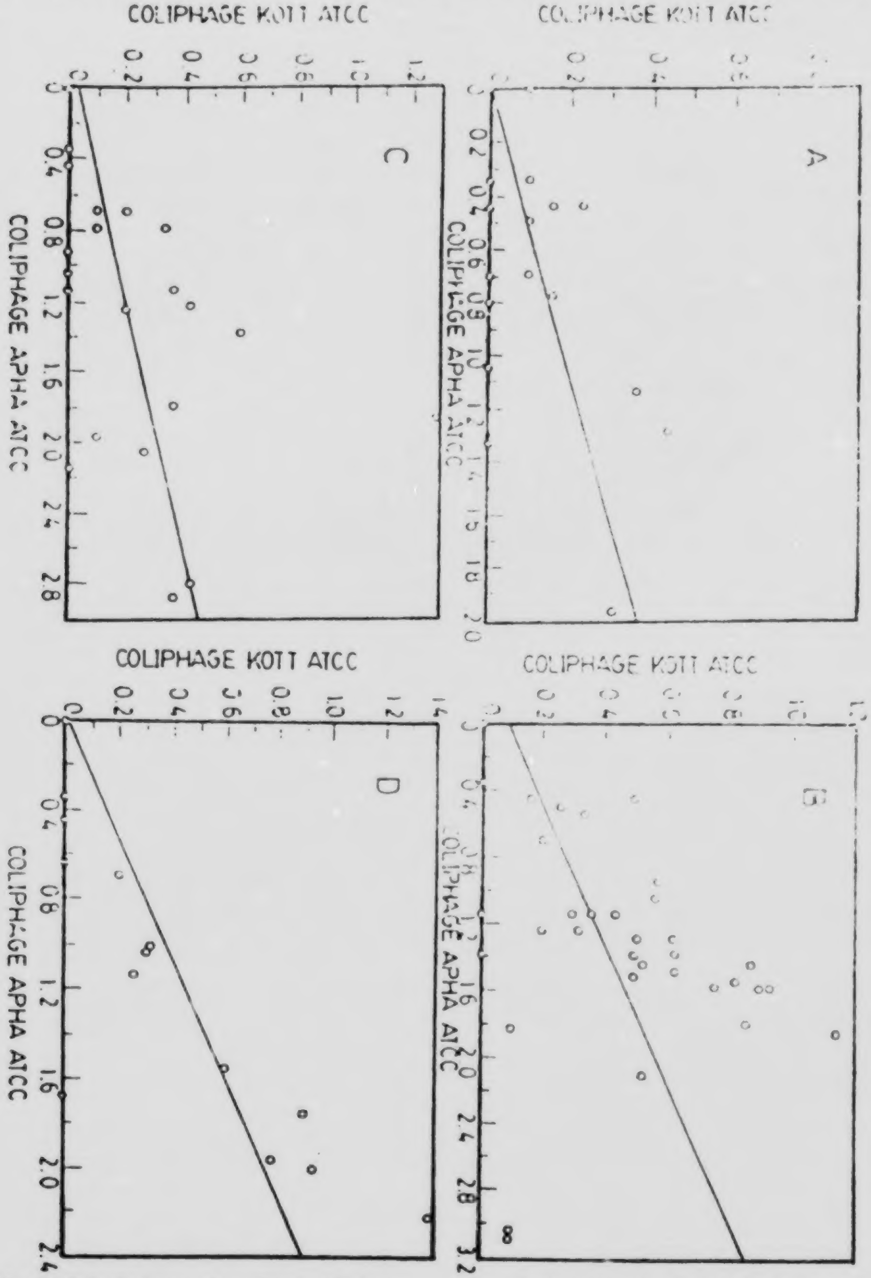
B Kott-MPN Coliphage (ATCC-ATCC)
$Y = 0.110 x + 1.590$ ($r^2 = 0.009$)

C Kott-MPN Coliphage (Local-ATCC)
$Y = 0.024 x + 1.590$ ($r^2 = 0.000$)

D Kott-MPN Coliphage (Local-Local
$Y = 0.001 x + 1.538$ ($r^2 = 0.000$)

FIGURE 2

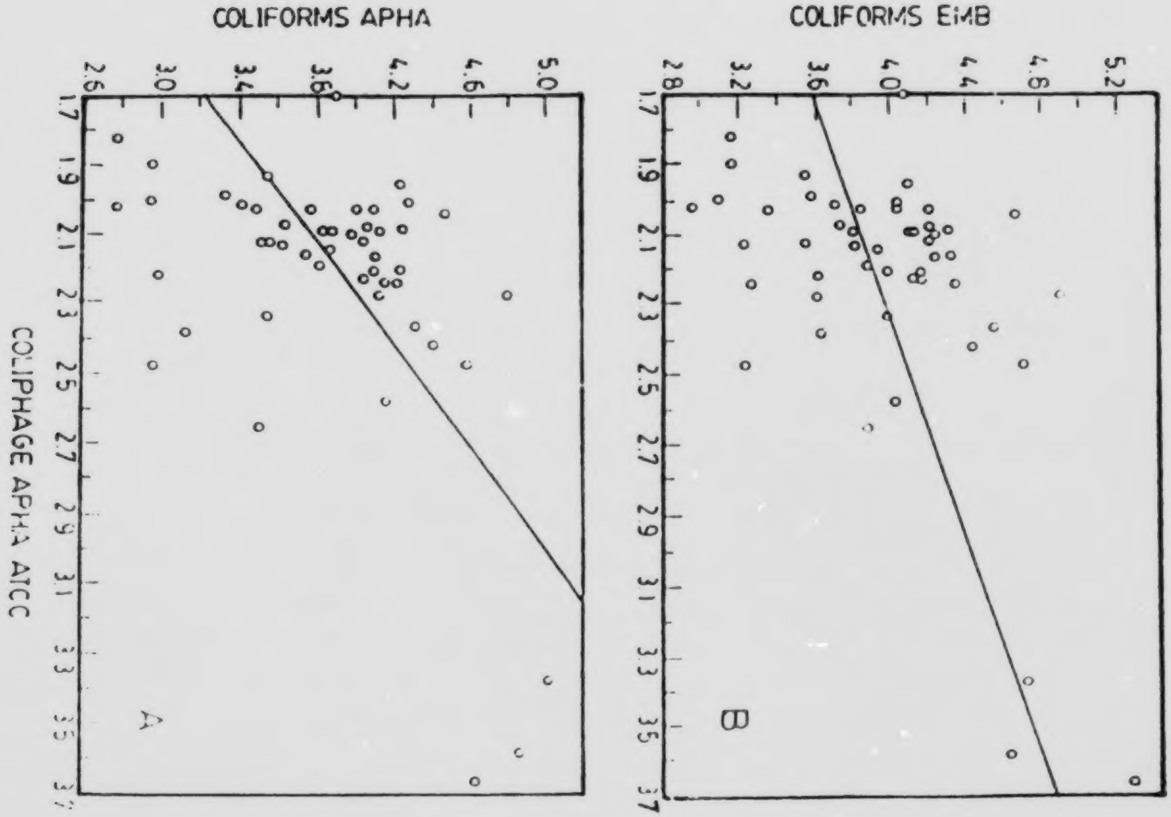
CORRELATION BETWEEN APHA AND KOTT-MPN COLIPHAGE PROCEDURES IN VARIOUS TYPES OF DRINKING WATER SAMPLES USING ATCC HOST



A	Kott-MPN Coliphage in Tap Water Y = 0.162 x + 0.016 (r^2 = 0.330)	C	Kott-MPN Coliphage in Storage Tanks Y = 0.171 x + 0.003 (r^2 = 0.409)
B	Kott-MPN Coliphage in Well Water Y = 0.245 x + 0.097 (r^2 = 0.313)	D	Kott-MPN Coliphage in Bottled Water Y = 0.348 x + (-0.025) (r^2 = 0.644)

FIGURE 3

CORRELATION BETWEEN COLIPHAGE COUNTS AND TOTAL COLIFORM PROCEDURE IN RAW WATER SAMPLES

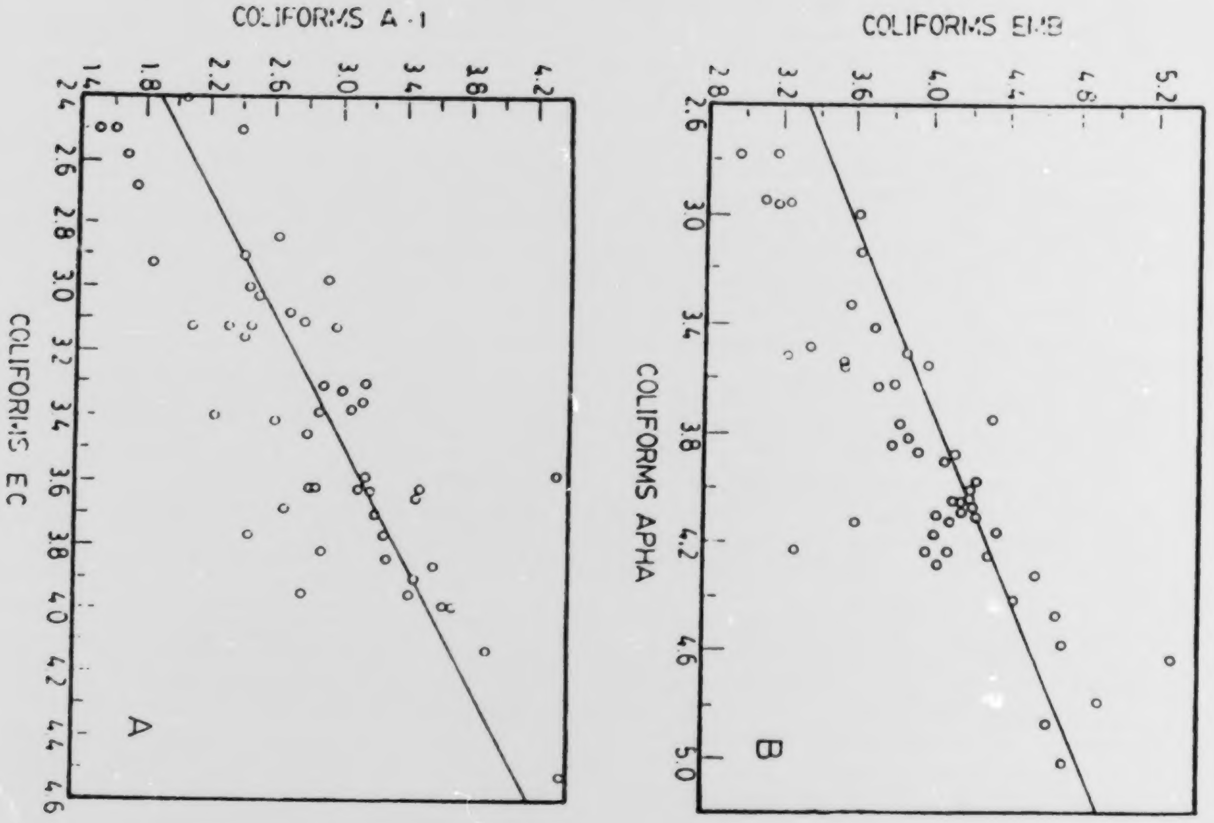


A **APHA-MPN Total Coliform**
$Y = 0.577x + 2.597$ ($r^2 = 0.192$)

B **EMB-MPN Total Coliform**
$Y = 0.579x + 2.685$ ($r^2 = 0.212$)

FIGURE 4

CORRELATION BETWEEN COLIFORMS PROCEDURES
IN RAW WATER SAMPLES



A EMB-MPN Total Coliform
$Y = 0.676x + 1.352$ ($r^2 = 0.501$)

B A-1-MPN Faecal Coliform
$Y = 1.024x + (-0.630)$ ($r^2 = 0.573$)

EVALUATION OF COLIPHAGE AND PRESENCE/ABSENCE TESTS FOR THE SANITARY CLASSIFICATION OF THE WATER RESOURCES AND THE QUALITY OF DRINKING WATER IN MOROCCO

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ABSTRACT

With partial support from the International Development Research Centre, the water quality control laboratory of the National Drinking Water Office (ONEP) conducted a study between 1986 and 1988 to evaluate the coliphage and the Presence/Absence (P/A) tests as simple and inexpensive tools for the sanitary classification of drinking water sources and the control of the microbiological quality of drinking water.

All over the country, 50 water sources comprising 40% of surface waters, 25 outlets of treatment or desalination plants and 25 locations inside water distribution networks were sampled in triplicate, three times each. Total and faecal coliforms were enumerated along with the coliphage and P/A tests using APHA Standard Methods as well as the methods in use within the ONEP laboratories. Tests were adapted to the type of sample examined.

For treated waters, the very low number of positive results, including those from coliphage and P/A tests, make it difficult to compare the analytical methods. The P/A test seems, however, to be well adapted for the control of the bacteriological quality of water for large systems of drinking water production or distribution, while the sensitivity of the APHA coliphage test needs to be improved before adopting it as a routine test to assess the microbiological quality of drinking water in Morocco.
Even for raw waters, the coliphage test proved not sensitive enough to detect the microbial contamination of either surface or ground waters, when compared to total or faecal coliforms tests.

Some considerations about the statistical analyses of microbiological data generated during the study are given in an annexed paper.

INTRODUCTION

In Morocco, as in most countries, the water quality control aimed to the assessment of faecal contamination is based on the bacteriological methods like the coliform test.

Coliform organisms are the hosts for coliphages and a relationship between coliform bacteria and coliphages which infect E. coli was established (Kenard and Valentine, 1974; Wentsel et al, 1982; Dutka et al, 1987) allowing the prediction of the degree of faecal contamination from the bacteriophages enumeration.

The Presence/Absence test (P/A test) is a simplification of the conventional standard fermentation tube procedure (FT) for total coliforms in drinking water. The test was proposed by Clark (1980) as a less expensive, more sensitive and easier method for monitoring drinking and raw water supplies.

The objective of the project on water quality control supported by IDRC is the evaluation of the coliphage and the Presence/Absence tests as simple, rapid and inexpensive methods for the sanitary classification of the water resources and the quality of drinking water in Morocco.

The country had by the end of 1987 an estimated population of 23.4 million inhabitants, 54.9% of them living in rural areas. Despite the rapid growth of the population (around 2.6% per year), the general sanitary indicators have improved rather rapidly as life expectancy rose from 47 years in 1960 to 61.6 years in 1987, and infant mortality decreased during the same period from 149 to 81 per thousand. Water-borne diseases continue, however, to play a major role in the general mortality, accounting for 35% during the period 1980 to 1985.

The development of water supplies has been rather rapid in Moroccan urban areas; one can consider, in fact, that all the urban population of the country has reasonable access to safe drinking water. In rural areas however, the situation is less satisfactory, except in villages that are close to large cities or are on the way of newly established water supply schemes for cities, as the present policy favours the integration of the water supply of these rural centres into the urban water supply projects. The global situation of water supply and sanitation is described in **Figure 1**, indicating the progress of the international drinking water and sanitation decade in the country.

The water quality standards that have been prepared in Morocco are derived from WHO guidelines for drinking water quality for all that concerns bacteriological and toxic parameters; for the rest of the parameters, several other sources have been used, especially EEC, U.S. and Canadian standards. For some aesthetical aspects, however, local considerations dictate the limit values adopted.

The regulations under approval make a distinction between the "control" done by the Government (i.e. the Ministry of Public Health) and the "self-control" to be performed by the drinking water supply undertakings.

To control about 70% of the urban potable water production and 17% of the distribution, L'Office National de l'Eau Potable (ONEP) has performed bacterial analyses on some 7,000 samples of treated waters and 1,200 samples of raw waters in its central and branch laboratories. The controls made by ONEP indicate a quite good bacteriological quality of drinking water in urban areas; in 1988 those controls revealed that less than 1.5% of the samples analyzed contained total coliforms per 100 mL.

In 1987, the National Hygiene Institute and a network of more than 20 regional laboratories of epidemiological survey and environmental health, analyzed 5,622 water samples for the Ministry of Health (including samples from rural areas) and also for certain municipal potable water distribution agencies. All the other laboratories, comprising those of some of the distribution agencies and a private laboratory, do not probably analyze more than do the National Hygiene Institute.

The number of analyses required to generalize the bacteriological quality control to the rural areas is of course far higher than all that have been done until now. It is felt, therefore, that the generalization of the controls to the rural areas will increase the need for a simple proven method for the evaluation of the bacteriological water quality.

MATERIALS AND METHODS

Fifty raw water sources and 50 drinking water sampling locations were selected in 41 centres spread all over the country.

The raw water sources consisted of: Boreholes (16); Wells (8); Springs (6); Reservoirs (6); Canals (4); and Rivers (10).

The drinking water sampling locations were: Surface water treatment plant outlets (5); Brackish or sea water desalination plant outlets (8); Entries of distribution systems (17); and Inside the distribution networks (25).

The procedure described in section 919C APHA Standard Methods (1985) with the addition of 2,3,5 triphenyl tetrazolium chloride and using _E_. _coli_ C (ATCC 13706) as host was used in this study to enumerate the coliphages. This _E_. _coli_ strain was kindly sent to the project team by Dr. Dutka (Canada Centre for Inland Waters (CCIW), Burlington, Ontario, Canada).

Raw water samples were subjected to the APHA Standard Methods (1985) faecal coliform test using the A-1 broth 5-tube MPN procedure.

Both raw and treated water samples were also subjected to the following APHA Standard Methods (1985) total and faecal coliform tests: the 5-tube MPN procedure using lauryl tryptose broth and brilliant green lactose bile broth for the confirmation of total coliforms, with faecal coliform confirmation in EC broth. For both raw and treated waters, hydrophobic square-grid membrane filters marketed as Iso-grid (WA Laboratories, Toronto, Ontario, Canada) were also used with Endo agar to estimate total coliforms, and with M-FC agar to estimate faecal coliform concentrations. All treated water samples were tested by the APHA Standard Methods P/A broth test.

For the A-1 broth procedure, only one incubation temperature was used (37°C until February 1988, and 44°C onwards) during a 24-hour period instead of 3 hours at 35°C and 21 hours at 44.5°C as indicated in the APHA Standard Methods. On the other hand, the incubation temperature for all total coliform and coliphage tests was 37°C instead of 35°C as indicated by APHA standards for those tests.

Waters were also subjected to the tests in use by the Moroccan National Drinking Water Office Central Laboratory for total and faecal coliforms for raw waters, a 3-tube MPN procedure was employed using lauryl tryptose broth and brilliant green lactose bile broth for the confirmation of total coliforms, and with faecal coliform confirmation on EC medium. In the same manner, treated waters were tested for total and faecal coliforms using tergitol-7 agar, and for faecal streptococci using Slanetz agar with confirmation on Roth broth. For both tests, Millipore or Sartorius 0.45 micrometer membrane filters were used.

A large part of the positive confirmation tests for total and faecal coliforms were subjected to isolation on MacConkey agar and identification by IMVIC procedure or the procedure marketed as the API test.

For all raw and treated water samples, pH, temperature, conductivity, turbidity and hardness were measured following the procedures in use in the ONEP central laboratory (all derived from APHA standards).

Free residual chlorine was measured in treated water samples using the orthotolidine method. The procedures are summarized in **Figure 2** for raw waters and **Figure 3** for treated waters.

RESULTS AND DISCUSSION

For the 3-tube and 5-tube MPN tests, values are reported as zero whenever no positive tubes were obtained.

Drinking Waters

Out of 3,354 presumptive tests performed on 153 samples (analyzed in triplicate), 50 tests (1.49% of the total number) led to positive results (see **Table 1**).

Out of the 16 positive presumptive tests that were submitted to confirmation and/or identification procedures, only three proved to be positive for total and faecal coliforms or for faecal streptococci.

The very low number of positive results makes it difficult to compare the analytical methods. It is, however, obvious from the results that in the conditions of the study, the coliphage test is by no means more sensitive than the other tests used. Its sensitivity needs certainly to be increased.

Raw Waters

Some considerations about the statistical analysis of the microbiological data within the project are developed in **Annex 1**.

The following analysis of data is only preliminary and concerns the median of the values obtained for each parameter for a given sample (analyzed in triplicate). In order to simplify the comparison of the results for different types of waters, the results are described for each parameter with only three variables: the first quartile, the third quartile and the median.

Figure 4 gives this type of description for the physicochemical parameters tested. Results indicate that the greatest part of the dispersion within raw waters occurs for the conductivity and hardness in ground waters, and for turbidity in surface waters.

Examination of **Figure** 5 gives the impression that the bacteriological quality of raw waters is quite good, while results reported in **Figures** 6 and 7 show clearly that in fact two entirely different situations occur:

- ground waters, for which the bacterial quality is excellent **(Figure 6)**;
- surface waters, for which the bacterial counts are far more dispersed and much higher **(Figure 7)**.

Contingency tables have been prepared to compare the ability of the quantitative and qualitative methods in detecting contamination (Presence/Absence, total and faecal coliforms, and coliphages).

As the frequency of contamination is expected to be quite different for ground waters and surface waters, the results obtained in each case are analyzed separately.

For surface waters, the different bacterial methods used give generally similar results in indicating the presence of contamination (see **Figure 8**). This may be simply attributed to the fact that those waters are heavily contaminated. On the other hand the coliphage test appears to be less reliable in detecting contamination than all the bacterial tests used (see **Figure 9**).

For ground waters, this type of comparison is more relevant, as positive and negative results of the bacterial tests are close to each other. **Figure 10** shows that the different bacterial tests give the same picture of contamination; the presumptive tests (LTS 5 and LTS 3) giving, as expected, higher estimates of contamination than the confirmation tests. But when the coliphage test is considered (see **Figure 11**), the lack of association between this test and the bacterial tests (including those determining faecal coliforms) becomes evident. In the conditions of the study, coliphage enumerations proved then to be less sensitive in detecting contamination of either surface or ground waters when compared to the bacterial enumeration techniques of total and faecal coliforms.

CONCLUSIONS AND RECOMMENDATIONS

On the basis of the results obtained in the study, the following preliminary conclusions may be drawn.

1. Treated Waters

- In treated waters, the overall contamination is very low.
- No conclusion can be drawn concerning the coliphage or P/A tests. The P/A test seems, however, to be well adapted for the control of large production units or large distribution networks.
- Very few presumptive positive results have been confirmed, confirmation tests are then necessary and provisions have been made to include a confirmatory step in the Moroccan standard method using MPN or membrane filtration procedures.

It is recommended to test the P/A and H_2S procedures in unchlorinated wells and boreholes (i.e. non-treated drinking waters).

2. Raw Waters

- The APHA LTS 5-tube MPN technique and the Moroccan LTS 3-tube MPN technique give very close estimates for total and faecal coliforms for the waters studied.

- The A-1 technique is sensitive for the detection of faecal coliforms, the technique is interesting as a potential standard test for the country because of the short time required and the non-necessity of confirmatory tests.

- The coliphage technique underestimated faecal contamination in surface and ground waters.

It is recommended to: increase the sensitivity of the coliphage test; and use the results of TC and FC techniques, along with an improved coliphage test for the sanitary classification of water resources.

ACKNOWLEDGEMENT

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TABLE I

TYPOLOGY OF POSITIVE SAMPLES OF TREATED WATERS

PRESUMPTIVE TEST											CONFIRMATIVE TEST							IDENTIFICATION TEST						
Sample	Number of tests	L.T.S. 5 MPN	P/A	Tergitol 7 37°C MF	- mFC MF	Tergitol 7 44°C MF	Iso-grid Endo MF	Slanetz MF	Coliphage	Number of + samples	Number of tests	LTS on BGB	P/A on BGB	LTS on EC	P/A on EC	on Litsky (from Slanetz)	Number of + samples		Iso-grid					Chlorine mg/1
1	22	+++	---	+++	+--	+--	---	+--	-	9	3	-		-		+	1							0.20
2	24	+++	---	+++	+--	++-	---	+++	---	12	3	+		+		-	2							0
3	24	---	---	+++	+--	+++	+++	---	++-	12	0						0		2					0.80
4	24	---	---	+--	---	---	---	---	---	1	0						0							1.0
5	24	---	---	---	---	---	---	+--	---	1	1					-	0							-
6	24	---	+++	---	---	---	---	---	---	3	2		-		-		0							-
7	7	-	-	-		-	-	-	+	1	0						0							-
8	24	+--	---	---	++-	---	+++	---	---	6	2	-		-			0							
9	18	+--	---	+++		+--		---	---	5	2	-		-			0							

FIGURE 1

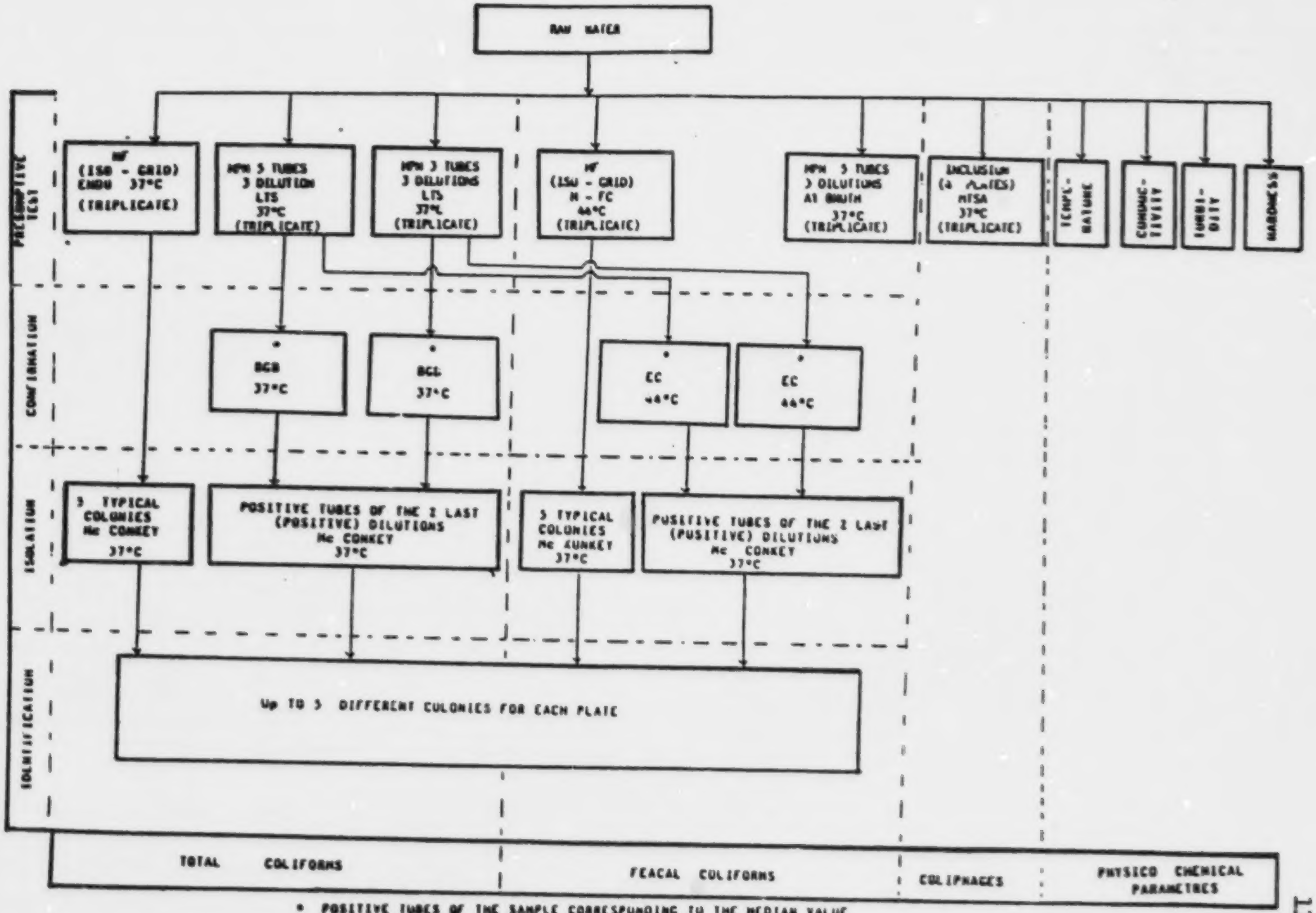
WATER SUPPLY AND SANITATION COVERAGE

	Percent of Population Served 1987	 1990 TARGET
In urban areas:		
Drinking Water Supply:		
- Water Connections	73%	80%
- Standpipes	27%	20%
Sanitation:		
- Connections to the Sewerage	34%	50%
- Pits and Septic Tanks	42%	50%
In rural areas:		
- Drinking Water Supply	11%	50%
- Sanitation	N/A*	50%

* N/A: Not Available

FIGURE 2

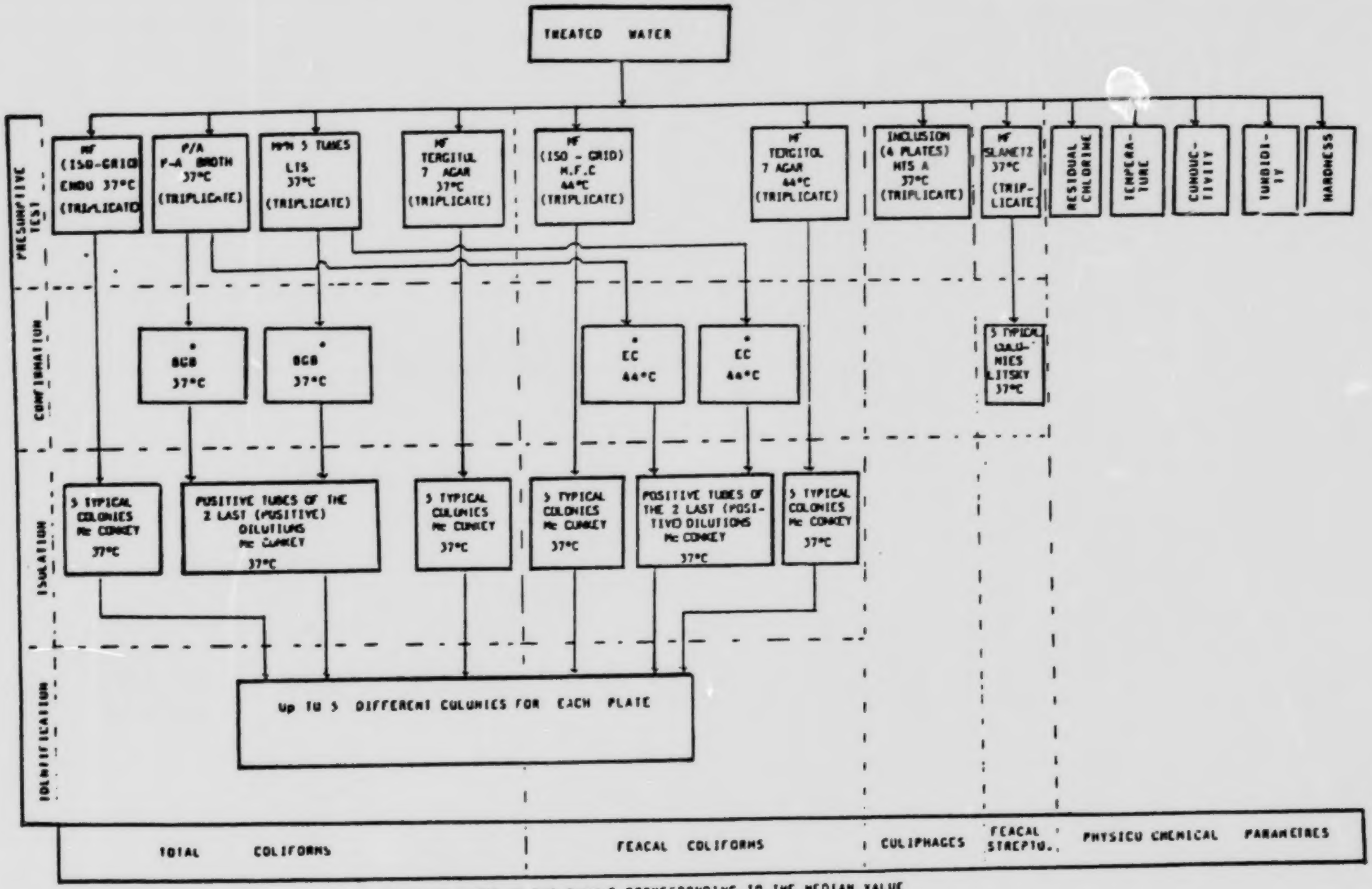
SCHEMATIC DIAGRAM OF RAW WATER EXAMINATION


RAW WATER
PRESUMPTIVE TEST
CONFIRMATION
ISOLATION
IDENTIFICATION
MF (ISO - GRID) ENDO 37°C (TRIPLICATE)
MPN 5 TUBES 3 DILUTION LTS 37°C (TRIPLICATE)
MPN 3 TUBES 3 DILUTIONS LTS 37°C (TRIPLICATE)
MF (ISO - GRID) M - FC 44°C (TRIPLICATE)
MPN 5 TUBES 3 DILUTIONS AT BROTH 37°C (TRIPLICATE)
INCLUSION (4 PLATES) MTSA 37°C (TRIPLICATE)
TEMPERATURE
CONDUCTIVITY
TURBIDITY
HARDNESS
* BGB 37°C
* BGL 37°C
* EC 44°C
* EC 44°C
5 TYPICAL COLONIES Mc CONKEY 37°C
POSITIVE TUBES OF THE 2 LAST (POSITIVE) DILUTIONS Mc CONKEY 37°C
5 TYPICAL COLONIES Mc CONKEY 37°C
POSITIVE TUBES OF THE 2 LAST (POSITIVE) DILUTIONS Mc CONKEY 37°C
Up TO 5 DIFFERENT COLONIES FOR EACH PLATE
TOTAL COLIFORMS
FEACAL COLIFORMS
COLIPHAGES
PHYSICO CHEMICAL PARAMETRES

* POSITIVE TUBES OF THE SAMPLE CORRESPONDING TO THE MEDIAN VALUE (POSITIVE TUBES OF THE POSITIVE SAMPLE WHEN ALONE).

FIGURE 3

SCHEMATIC DIAGRAM OF TREATED WATER EXAMINATION


TREATED WATER
PRESUMPTIVE TEST
CONFIRMATION
ISOLATION
IDENTIFICATION
MF (ISO-GRID) ENDO 37°C (TRIPLICATE)
P/A P-A BROTH 37°C (TRIPLICATE)
MPN 5 TUBES LTS 37°C (TRIPLICATE)
MF TERGITOL 7 AGAR 37°C (TRIPLICATE)
MF (ISO - GRID) M.F.C 44°C (TRIPLICATE)
MF TERGITOL 7 AGAR 44°C (TRIPLICATE)
INCLUSION (4 PLATES) MTS A 37°C (TRIPLICATE)
MF SLANETZ 37°C (TRIPLICATE)
RESIDUAL CHLORINE
TEMPERATURE
CONDUCTIVITY
TURBIDITY
HARDNESS
* BGB 37°C
* BGB 37°C
* EC 44°C
* EC 44°C
5 TYPICAL COLONIES LITSKY 37°C
5 TYPICAL COLONIES Mc CONKEY 37°C
POSITIVE TUBES OF THE 2 LAST (POSITIVE) DILUTIONS Mc CONKEY 37°C
5 TYPICAL COLONIES Mc CONKEY 37°C
5 TYPICAL COLONIES Mc CONKEY 37°C
POSITIVE TUBES OF THE 2 LAST (POSITIVE) DILUTIONS Mc CONKEY 37°C
5 TYPICAL COLONIES Mc CONKEY 37°C
Up TO 5 DIFFERENT COLONIES FOR EACH PLATE
TOTAL COLIFORMS
FEACAL COLIFORMS
COLIPHAGES
FEACAL STREPTO.
PHYSICO CHEMICAL PARAMETRES

* POSITIVE TUBES OF THE SAMPLE CORRESPONDING TO THE MEDIAN VALUE (POSITIVE TUBES OF THE POSITIVE SAMPLE WHEN ALONE).

FIGURE 4

RAW WATERS: DIAGRAM OF THE 1st and 3rd QUARTILES AND THE MEDIAN FOR PHYSICO-CHEMICAL PARAMETERS

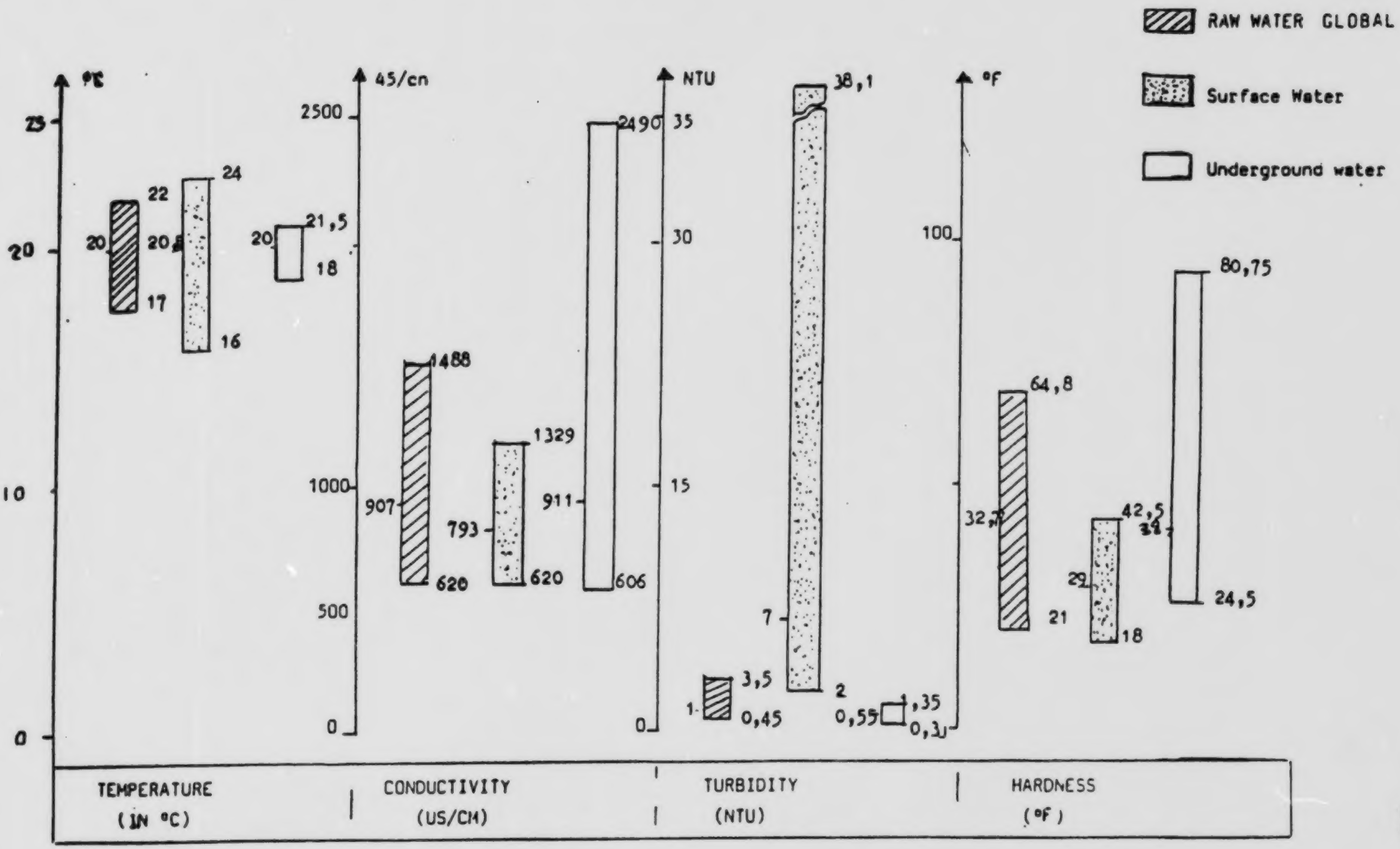


Fig. n° 5

DIAGRAM OF THE 1st AND 3rd QUARTILES AND MEDIAN

(Raw Waters)

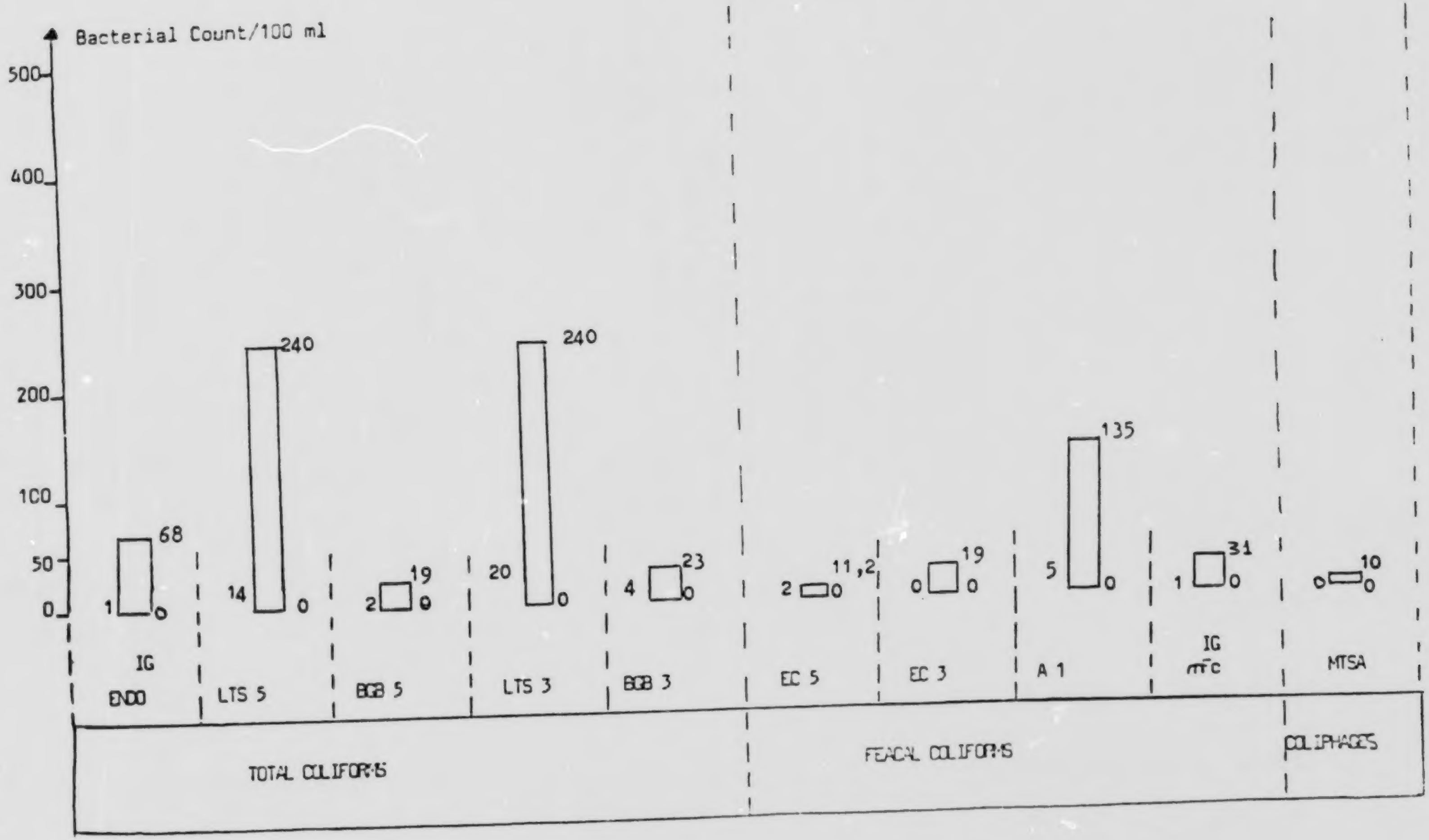


Fig n° 6

DIAGRAM OF THE 1^{st} AND 3^{rd} QUARTILES AND MEDIAN

(Surface Waters)

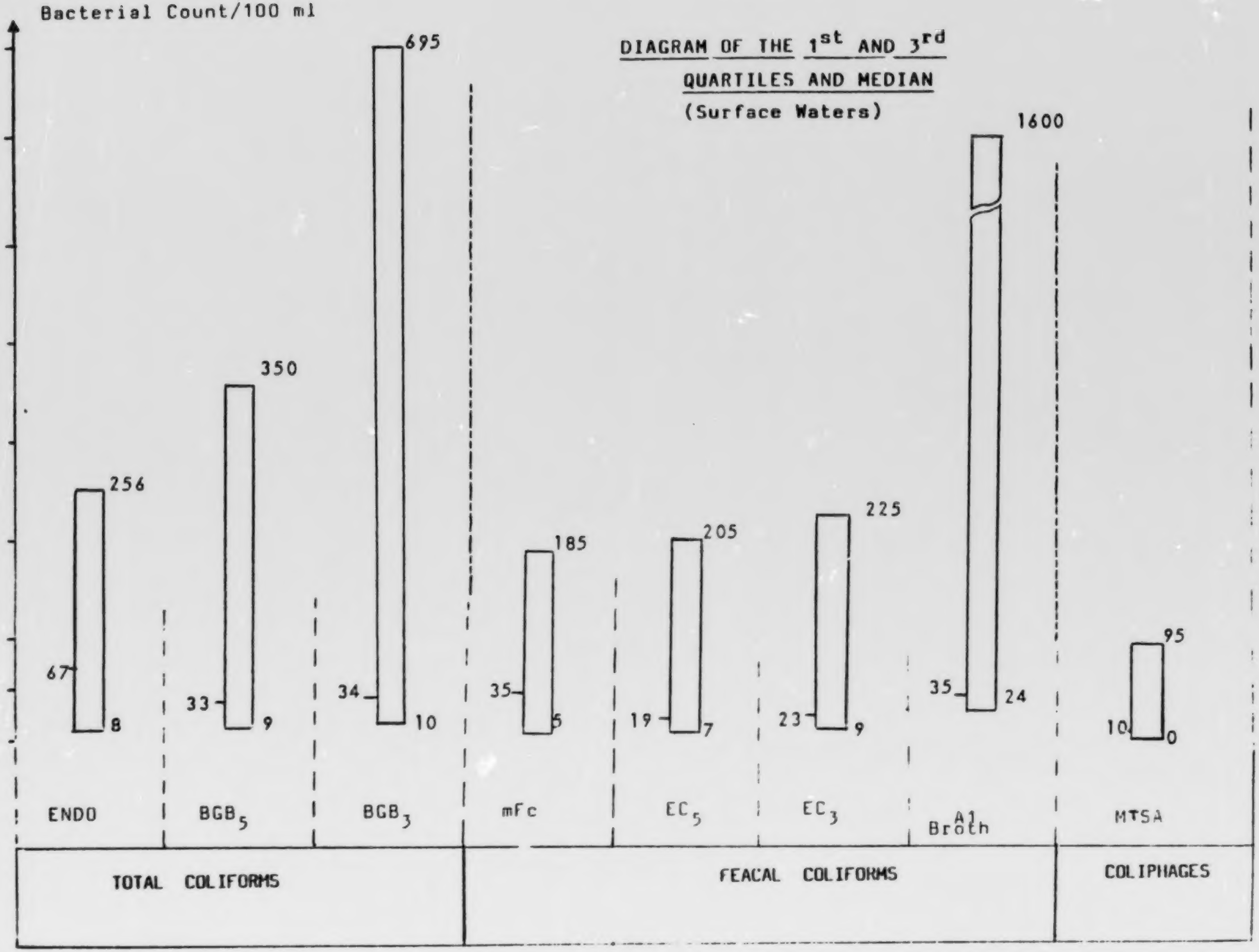


Fig. n° 7

DIAGRAM OF THE 1st AND 3rd QUARTILES AND MEDIAN

(GROUND WATERS)

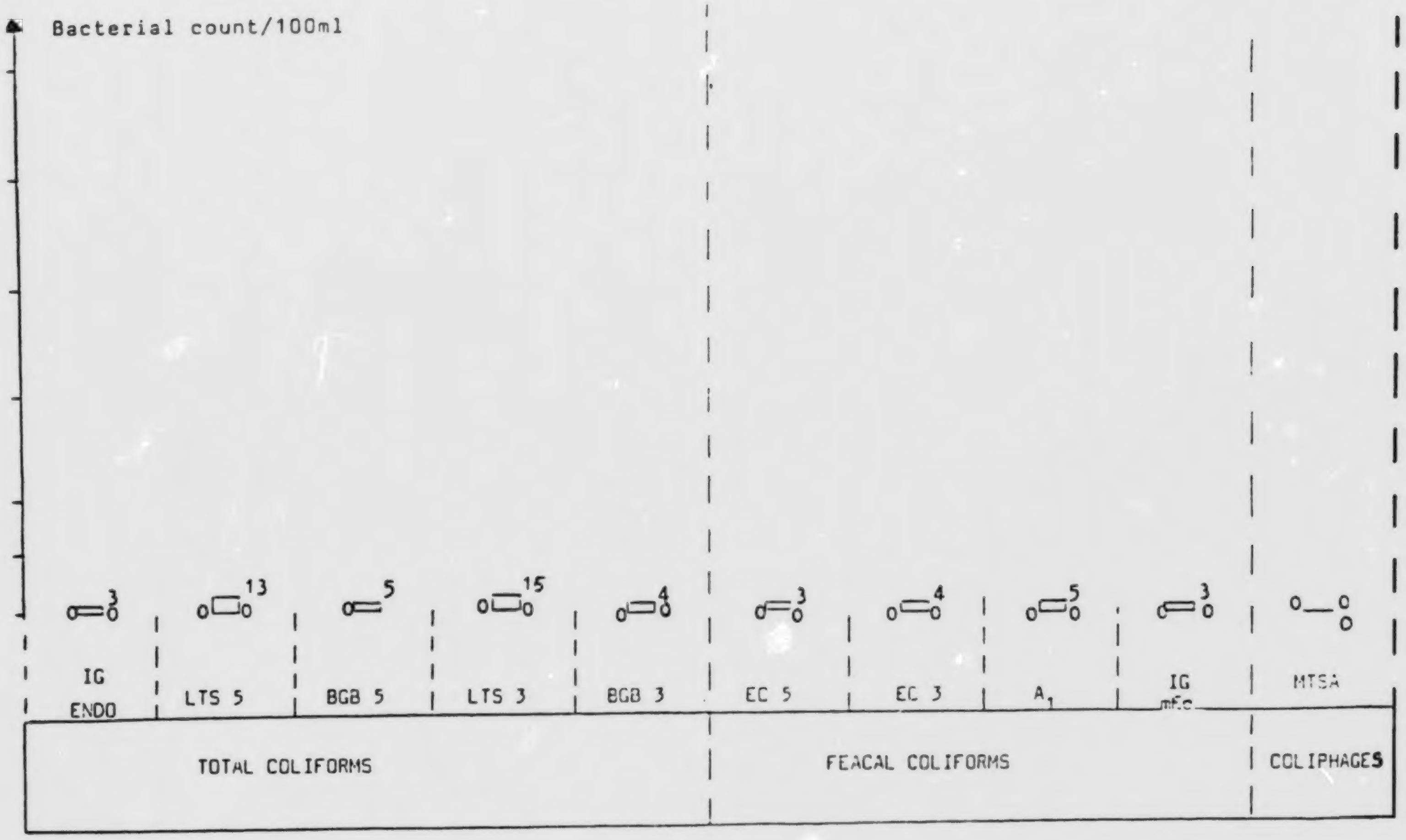


FIGURE 8

CONTINGENCY TABLES (SURFACE WATERS)

		+ MFC		
	+	47	2	49
ECm5				
	-	3	0	3
		50	2	52

		+ ECm3	-	
	+	53	2	55
ECm5				
	-	0	4	4
		53	6	59

		+ ENDO	-	
	+	43	3	46
ECm5				
	-	2	1	3
		45	4	49

		+ BGR 5	-	
	+	52	3	55
ECm5				
	-	4	1	5
		56	4	60

		+ BGR 3	-	
	+	54	3	57
ECm5				
	-	4	1	5
		58	4	62

		+ LTS 5	-	
	+	55	0	55
ECm5				
	-	5	0	5
		60	0	60

		+ LTS 3	-	
	+	55	0	55
ECm5				
	-	5	0	5
		60	0	60

FIGURE 9

CONTINGENCY TABLES (SURFACE WATERS)

ECm5

		+	-	
Coliphage	+	36	2	38
	-	18	4	22
		54	6	60

ECm3

		+	-	
Coliphage	+	34	3	37
	-	13	5	18
		47	8	55

A-1 Broth

		+	-	
Coliphage	+	33	2	35
	-	20	2	22
		53	4	57

ENDO

		+	-	
Coliphage	+	29	2	31
	-	15	2	17
		44	4	48

mFC

		+	-	
Coliphage	+	33	1	34
	-	16	1	17
		49	2	51

LTS 3

		+	-	
Coliphage	+	37	0	37
	-	22	0	22
		59	0	59

LTS 5

		+	-	
Coliphage	+	37	0	37
	-	22	0	22
		59	0	59

BGB 5

		+	-	
Coliphage	+	36	1	37
	-	1	3	4
		37	4	41

BGB 3

		+	-	
Coliphage	+	36	1	37
	-	19	4	23
		55	5	60

FIGURE 10

CONTINGENCY TABLES (GROUND WATERS)

		+ MFC	-	
ECm5	+	23	12	35
	-	8	51	59
		31	63	94

		+ ECm3	-	
ECm5	+	32	6	38
	-	4	56	60
		36	62	98

		+ ENDO	-	
ECm5	+	24	10	34
	-	3	54	57
		27	64	91

		+ BGB 5	-	
ECm5	+	35	2	37
	-	10	52	62
		45	54	99

		+ BGB 3	-	
ECm5	+	32	4	36
	-	3	53	56
		35	57	92

		+ LTS 5	-	
ECm5	+	35	1	36
	-	14	48	62
		49	49	98

		+ LTS 3	-	
ECm5	+	34	3	37
	-	14	48	62
		48	51	99

FIGURE 11

CONTINGENCY TABLES (GROUND WATERS)

		ECm5 +	ECm5 -	
Coliphage	+	18	5	23
Coliphage	-	15	57	72
		33	62	95

		ECm3 +	ECm3 -	
Coliphage	+	14	8	22
Coliphage	-	20	54	74
		34	62	96

		A-1 Broth +	A-1 Broth -	
Coliphage	+	15	7	22
Coliphage	-	22	53	75
		37	60	97

		ENDO +	ENDO -	
Coliphage	+	13	6	19
Coliphage	-	13	58	71
		26	64	90

		mFC +	mFC -	
Coliphage	+	18	2	20
Coliphage	-	13	59	72
		31	61	92

		LTS 3 +	LTS 3 -	
Coliphage	+	20	2	22
Coliphage	-	28	50	78
		48	52	100

		LTS 5 +	LTS 5 -	
Coliphage	+	20	2	22
Coliphage	-	29	48	77
		49	50	99

		BGB 5 +	BGB 5 -	
Coliphage	+	18	3	21
Coliphage	-	27	51	78
		45	54	99

		BGB 3 +	BGB 3 -	
Coliphage	+	16	5	21
Coliphage	-	26	50	76
		42	55	97

ANNEX 1: PRELIMINARY CONSIDERATIONS ABOUT THE STATISTICAL ANALYSIS OF MICROBIOLOGICAL DATA WITHIN IDRC WATER QUALITY CONTROL PROJECTS

1. The Objective:

Compare different methods of determination of:

- Total coliforms: Iso-grid (MF-Endo), BGB5 (MPN5), BGB3 (MPN3);
- Faecal Coliforms: Iso-grid (MF-MFC), EC5 (MPN5), EC3 (MPN3), A-1 (MPN5);
- Coliphages.

Different analytical techniques:

- Membrane filtration: Iso-grid;
- MPN 5-tube: BGB5, EC5, A-1;
- MPN 3-tube: BGB3, EC3.

As overall relations, one can expect that total coliform counts be higher than faecal coliform counts. (The ill precision of subsampling and counting estimations may, however, lead to a more complex situation.)

For coliphages, the relationship is more difficult to establish as it is possible to find coliphages without encountering coliforms; the reverse being also possible.

Note: The LTS5 and LTS3 are not considered here as they are only presumptive tests for coliforms.

2. Left Censoring:

All the results are left censored by the lower detection limit which, in this case, is different for the different methods, the volumes used in the different tests being different as well.

TEST	VOLUME OF WATER USED mL	APPROXIMATE LOWER DETECTION LIMIT IN COUNTS/100mL
Iso-grid (MF)	100	1
MPN (5-tube)	5x10+5x1+5x0.1=55.5	2
MPN (3-tube)	3x10+3x1+3x0.1=33.3	3
Coliphages	4x5=20	5

The question may arise as to whether, in the absence of more sophisticated ways of estimation, it would be relevant to add to the series random numbers (from which distribution?) comprised between zero and the detection limit of the method to replace the non-detects.

But another question is: for the methods measuring the same bacterial group (total coliforms and faecal coliforms), what will be the effect of replacing the non-detects of one technique with the actual results obtained by a more sensitive technique when these results are measurable and are less or equal to the detection limit of the less sensitive method?

3. Right Censoring:

The techniques used for bacterial counts have different upper counting limits. These are dependent on the technique itself and on the volume of water and the dilution used. For the standard tests, the limits are:

TESTS	UPPER COUNTING LIMIT
Iso-grid membrane filtration	**9,229**
MPN (5-tube)	**1,609**
MPN (3-tube)	**1,100**
Coliphages	**200**

Here the concerns are more important because the counts being higher, their weight in the computation of the correlation coefficients and in the regression equations is significant.

For the determination of the median and the third quartile (if not beyond the upper counting limit), it is important to use all the data to avoid biasing these parameters.

However, for the comparison of different methods (either by parametric or non-parametric tests), the values exceeding the upper counting limits should be either discarded or estimated.

Another aspect deals with the fact that, at least in the Moroccan project, some determinations have been made with higher upper counting limits using more dilutions. These limits are:

TEST	INCREASED UPPER COUNTING LIMIT
Iso-grid membrane filtration	92,290
MPN (5-tube)	16,090 or 160,900
MPN (3-tube)	11,000 or 110,000
Coliphages	2,000 or 20,000

On one hand, the results of these tests disturb the distribution and play a major role in the computation of the correlation and regression coefficients. In a first approach the values (exceeding the "normal" upper counting levels) should be discarded. But on the other hand, it may be possible to use these values to estimate the "right tail" of the distribution and use it in some manner to estimate this tail for the whole series.

4. Tests Based on Ranking:

The tests based on ranking should concern only the results within a definite category (total or faecal coliforms), as one cannot expect these series to be the same when considering different populations. The comparison based on the ranking between the coliphages and total or faecal coliforms is not relevant.

5. Evaluation of the Reproducibility of Different Tests:

For two methods intended to measure the same parameter, the use of three subsamples leads to 6 series. How to evaluate and compare the reproducibility of the two methods (mean and variance)? What if only two replicates have been performed for some samples? Could that question be also treated in non-parametric terms?

6. The Data Analysis Procedure:

Summarizing Data

The box plots are convenient to summarize data. The logarithmic plots are the most illustrative, but the presence of numerous non-detects (zeros) necessitates a number ≥ 1 to be added to the data before the analysis.
For the non-detects, should that number be uniform (1 for example), or should it be a random number belonging to the interval: 1, 1+LDL (LDL being the lower detection limit)?

How to generate these numbers? From which distribution?

6.1 - Comparison Between Different Techniques

If the parametric statistical analysis (after solving the problems related to left and right censoring) produces good results (possibly after a log transformation), the regression between the different techniques is a valuable tool leading to the prediction of an estimate by one technique from the other. The original idea of the coliphage test (in contrast with P/A and H_2S tests) is that, from the results of this test, the total and faecal coliform counts could be predicted.

As far as the same population is concerned (total coliforms, faecal coliforms), the ranking techniques lead to the highest estimate of the bacterial counts. The Wilcoxon rank sum test seems to be an adequate test to that end. (Any other relevant test?)

6.2 - The Presence/Absence Aspect

For the treated waters, but also for untreated underground waters, the sharp left censoring of the results suggest the use of the probability of the detection of the bacterial contamination as a comparison factor among techniques (see El-Shaarawi, NWRI contribution 88).

Note: This will apply to P/A and H_2S test as probably the only approach besides contingency tables.

7. Data Analysis Strategy:

What should be really useful is to design a strategy in two steps as far as the left and right censoring are concerned:

i) a first approximation approach dealing in a very simple way with left and right censoring:

  - addition of some random variable (from which distribution?) to the non-detects;
  - non-consideration of the numbers beyond the upper counting limits, even when (through additional dilutions) there is data available;
  - the limitations of this approach are to be carefully defined.

ii) a second approximation using more sophisticated statistical tools may be used to cope with left and right censoring.

For both steps, the limitations, the techniques and possibly specific references to the minitab data analysis software features, need to be specified.

In addition, two questions may be raised:

a) The counting range being rather large, is the relation between the results obtained by different techniques valid over the entire detection range? Is it not more adequate to "stratify" the results? One way of doing this is to group water sources into surface and ground waters, but this has more to do with the nature of the water sources than with the capability of each bacteriological technique to produce a good estimate of bacterial counts.

b) How to deal with non-detects in the confirmation techniques (BGB and EC) from LTS presumptive tests?

DEVELOPMENT OF A FIELD TEST KIT FOR MICROBIOLOGICAL ANALYSIS OF WATER IN MALAYSIA

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ABSTRACT

The usefulness of coliphages for assessing the quality of water, and as indicators of faecal pollution in Malaysia was investigated. Comparative analyses between the APHA 919C method for coliphage enumeration and other standard techniques for coliform enumeration showed a strong correlation between the tests for the waters analyzed. Further investigation of the coliphage test showed that it was rapid and easy to use. Modifications to the APHA 919C method were made for the purpose of adapting it for use as a field kit. The modifications included: the omission of TPTZ for enhancing plaque visibility, the use of Gelrite in place of agar, the replacement of the frozen host for a host maintained in bacterial discs, and incubation at ambient temperature rather than at 35°C. A prototype field kit was developed and tested in the field for about six months. Tests showed the kit to be robust and reliable for coliphage enumeration of natural waters.

INTRODUCTION

In Malaysia, there are presently two major on-going interagency programmes at the national level concerned with water quality. The Department of Environment (DOE) undertakes a National Water Quality Monitoring Programme (NWQMP) with the principal objective of documenting the status and trend of natural water quality, while the Ministry of Health runs a National Drinking Water Quality Surveillance Programme (NDWQSP) principally to ensure the safety and acceptability of drinking water from public water supply systems. In addition, it also assists in the development of water supplies to rural communities.

Bacteriological testing is among the water quality tests included in the above-mentioned programmes. Standard techniques based on the detection of coliforms are normally used for bacteriological testing. However, current techniques are rather complex and expensive and require well equipped laboratories generally located in the larger urban areas. The problems of logistics related to samples taken from distant rural areas and the limited financial and manpower resources often only allow a limited programme to be implemented. Quite often visits to sampling sites are in response to particular problems (such as disease outbreaks), thus reducing the value of the programme.

The need for the development of more rapid low-cost techniques for the determination of bacteriological quality of water has been given much thought in Malaysia in light of the problems mentioned above. During the past two-and-a-half years, the use of coliphages as alternative indicators of faecal contamination was evaluated with the view to adapt the technique for application as a portable field kit. Extensive comparative tests were carried out to evaluate the APHA 919C method for coliphage enumeration against other techniques such as the conventional multiple tube MPN method, the A-1 broth MPN method and the M-FC membrane filter technique. Tests were also carried out to evaluate the hydrogen sulphide test described by Hazbun and Parker (1983).

This report presents the results of studies on the development of the field kit and testing carried out in the field on its robustness and acceptability.

MATERIALS AND METHODS

Coliphage Enumeration

The enumeration of coliphages was carried out following procedures described in the APHA 919C method (APHA, 1986). E. coli C (ATCC Culture No. 13706) was used as the bacterial host. Frozen E. coli C host for use in the test was allowed to thaw at room temperature and then warmed to 44.5°C for about five minutes.

In a modified procedure for adaptation into a portable field kit, a linear polysaccharide gelling agent, commercial name "Gelrite" from Scott Laboratories Inc., was used in place of agar. Gelrite was prepared separately as 0.75% solution in deionized water. Frozen E. coli C host was replaced by bacterial disc culture (BDC). The procedure for preparation involves the centrifugation of an 18 hour culture of E. coli C in Tryptic Soy Broth (TSB) and resuspension with an appropriate volume of sterilized evaporated milk to give a 20 times concentrated culture. A drop of the concentrated suspension was applied using a pasteur pipette onto sterile paper discs of about 6mm diameter. The discs were dried in a desiccator for 48 hours and subsequently stored in sterile containers at 4°C for later use. To resuscitate, discs were incubated in TSB at ambient room temperature for 18-24 hours.

Coliform Bacteria Detection and Enumeration

Standard bacteriological water quality tests such as the multiple tube fermentation MPN (McCrady, 1915), A-1 Broth MPN (Andrews and Presnell, 1972; Standridge and Delfino, 1981) and the membrane filtration M-FC (American Public Health Association, 1985) were performed together with the coliphage assay on the same water samples.

Apart from the comparative studies between the coliphage assay and the standard bacteriological methods for the determination of water quality, studies were also carried out on the hydrogen sulphide test. The technique as described by Hazbun and Parker (1983) was used except that 0.2g sodium dodecyl sulphate was used in place of Teepol which was no longer manufactured.

Other studies performed included:

I. effect of temperature of incubation on phage enumeration;

II. effect of exclusion of 2,3,5-triphenyl tetrazolium chloride (TPTZ) on phage enumeration;

III. effect of storage on phage enumeration;

IV. use of bacterial disc cultures (BDC) instead of frozen host *E. coli* C;

V. use of the linear polysaccharide gelling agent, Gelrite, in place of agar;

VI. interference by bacteriocinogenic bacteria on the coliphage test.

Water Samples

Water samples came from various sources such as open wells, tube wells, rivers, gravity feed water supply systems and small scale estate water supply systems. Sampling was carried out taking the standard precautions to avoid contamination during sampling. Preparation of samples for analyses for coliphages were normally carried out in the field and incubated at ambient temperature.

RESULTS

Results of the various studies carried out have been reported in detail in the Final Project Report submitted to IDRC (Mohd Sanusi et al, 1987) and elsewhere (Loh et al, 1988). The main findings are described here for clarity in understanding the discussions later in this paper.

Coliform-coliphage relationship

Coliform and coliphage concentrations in water samples were analyzed by linear regression analysis after log transformations of the data. Results showed high correlations between coliphage numbers and total and faecal coliforms, for both surface and well waters. The correlation improved with time of incubation for the coliphage test, with the best correlation at 24 hours. However, an 8 hour incubation time for the coliphage test was sufficient to give an indication of the presence of faecal contamination.

Coliphage concentrations were best correlated with total coliforms in surface waters, while for well waters, a better correlation was observed with faecal coliforms. This is indicative of the distribution of coliforms in different water sources. Surface waters probably contain significantly more environmental phages of non-faecal origin as opposed to ground waters.

While linear regression analyses of the log transformed data showed a significant correlation between coliphages and coliforms, results from some water samples deviated from this trend. For some of these samples, the absence of detectable coliphages did not necessarily correspond with the absence of coliforms. This was found to be true even up to coliform levels of about 3000/100 ml. On other occasions, high coliphages were detected while coliform numbers were low, as low as 43/100 ml.

Tests were also made with the prototype field kit for well samples to assess the coliform-coliphage relationship. Samples taken in the field were analyzed using the modified APHA 919C technique where Gelrite replaced agar, 2,3,5 triphenyl tetrazolium chloride (TPTZ) was not used, BDC cultures were used in place of frozen host cultures and samples were incubated at ambient temperature (range 25-35°C). Counts were made after about 18 hour incubation. At the same time M-FC counts for faecal coliforms were also carried out.

From 147 well water samples (open and tube wells) taken from various sources in the central region of the country, between October 1987 and March 1988, linear regression analyses of the log transformed data were made. Results showed that there was a significant correlation ($p<0.001$) between M-FC counts and coliphage counts (**Figure 1**). The correlation coefficient, r^2, was 0.4767.

In further tests carried out in the northern State of Kedah in the District of Kulim, correlation analyses for 118 dug-well and 116 tube-well samples showed significant correlations ($p<0.001$) between M-FC and coliphage concentrations (**Figures 2 and 3**). Values for r^2 were lower at 0.2775 and 0.1539 for dug-wells and tube-wells, respectively.

Effect of Temperature of Incubation

In adapting the coliphage test as a field kit the exclusion of an incubator would be an advantage. Tests were carried out to determine if the test could be done at variable temperatures and assess if differences between the results obtained were significant. Laboratory experiments were carried out for temperature ranges of 25° to 35° and at room temperature (27-31°C). The results showed that there were no significant differences ($p<0.05$) between phage enumeration at the temperature range 25° to

35° and that incubated at room temperature. It was noted, however, that higher counts were generally recorded at lower incubation temperatures. This observation was also noted in earlier experiments (Mohd Sanusi et al, 1987).

Use of bacterial disc cultures

The use of bacterial disc cultures (BDC) as an alternative to frozen host preparations in the coliphage test was examined. The procedure for preparation of such cultures was described earlier.

Experiments showed that E. coli C host could be successfully stored using the method of desiccation in the presence of protective evaporated milk colloids on an antibiotic disc. Experiments showed that E. coli C bacteria could be successfully resuscitated in Trypticase Soy Broth after four weeks of storage at 4°C.

Omission of TPTZ from test

It was found that while the addition of TPTZ enhanced plaque visibility, it was not essential. Using a black background to view the plates, the omission of the chemical did not affect significantly the enumeration of plaques.

Use of Gelrite in place of agar

The use of gelrite was investigated as an alternative to agar with the view to avoid the laborious task needed to maintain molten the Trypticase Soy Agar until use. Gelrite in a concentration of about 0.75% in deionised water was found to be suitable as a substitute for agar. A comparison of coliphage enumeration using agar and Gelrite showed that there was not significant difference between the two media used at 6, 8 or 24 hours incubation.

Effect of storage of water samples

Under standard procedures, water samples are kept at 4°C during storage prior to analysis. Samples are normally required to be analyzed within 24 hours after sampling. The effect of storage on the coliphage and coliform tests were examined for river samples. Results showed that coliphage numbers remained fairly constant even after a week of storage while coliform numbers declined with time of storage.

Interference by Bacteriocinogenic bacteria

Bacteriocinogenic bacteria were encountered which could interfere with coliphage enumeration since these gave false plaques. They were generally encountered in waters with high coliform numbers but absent in cleaner waters. Of 28 isolates confirmed as bacteriocinogenic bacteria, 17 were identified as Klebsiella pneumoniae, 7 were E. coli, and 2 each of Klebsiella oxytoca and

Enterobacter cloacae. Experience showed that "plaques" formed by bacteriocinogenic bacteria can be distinguished by a small but distinct colony in the dead centre of the clear zone.

Hydrogen sulphide test

Studies were made on the hydrogen sulphide test as described by Hazbun and Parker (1983) to assess its suitability as a simplified method for bacteriological water quality analysis. Results showed that the test was more suitable as a qualitative rather than a quantitative test. Spearman's Rank correlation analysis showed that the degree of blackening was significantly correlated with total and faecal coliform levels in the water tested. Correlation was greater for total rather than faecal coliforms and the correlation was greater at 48 hours rather than 24 hours. Of the total of 47 bacteria isolates from positive tests, confirmed to give positive reaction on reintroduction to new media, 40 were Citrobacter fruendii, 2 each of K. pnuemoniae and Enterobacter cloacae, and 1 each of Enterobacter aerogenes and the genus Kluyvera.

Coliphage field kit tests

The field kit which has a styrofoam box enclosed in canvas sheet, was used in the field over a six-month period and observations made on its robustness showed that it was strong enough and was not easily damaged. Glassware and media in the box were well protected and breakages were not recorded. In addition, the styrofoam box afforded protection to the sun and rain and temperatures within the box remained fairly constant and within the range for sample incubation. The styrofoam box, however, was the main component which showed most wear, as expected. Over the six-month period, wear was confined mainly to the edges of the box. During this period, over 600 samples were tested.

The kit was strong enough to be carried by hand, on a bicycle or in the trunk of a car. The kit measures 38cm x 25.5cm x 33.5cm and weighs about 6.4 kg. Effort is now being made to reduce the weight by substituting the use of glassware with that of plastic.

DISCUSSION

Results of tests carried out earlier with the APHA 919C method for coliphage enumeration showed that the method can be applied for bacteriological testing of Malaysian waters (Mohd Sanusi et al, 1987; Loh et al, 1988). Tests showed that there was significant correlation between coliphage enumeration by the method and coliforms enumerated by conventional techniques such as the Multiple tube MPN, A-1 MPN and the M-FC methods. Linear regression analyses for the methods using surface waters showed significant correlation ($p<0.0001$). The correlation improved with time of incubation with the best correlation at 24 hours. Recent results with well water also showed significant correlation between coliphage and faecal coliforms enumerated by the M-FC technique ($p<0.001$).

Further tests on the effect of exclusion of TPTZ, the effect of temperature of incubation, and the use of Gelrite in place of agar which were carried out showed that:

(a) TPTZ may be excluded from the test procedure without affecting plaque enumeration. Plates were found to be best viewed against a dark background as opposed to the use of a diffused white light source or a white background when TPTZ is used.

(b) The coliphage test could be carried out at ambient temperature within the range 25-35°C without significant effect on the test as opposed to the temperature of 35°C prescribed in Standard Methods for the APHA 919C test.

(c) The linear polysaccharide, Gelrite, may be a substitute for agar. Tests between the two media showed that coliphage enumeration was not affected by the use of Gelrite.

In addition, it was found that storage of water samples at 4°C had less effect on coliphages than on faecal coliforms, and that E. coli C host bacteria could be successfully stored in the desiccated form as bacterial discs.

Based on the above findings, a portable field kit has been designed with a capacity for eight tests. The prototype kit currently developed includes the media dispensed into McCartney bottles and contained in stainless steel trays, the bacterial host in BDC form, petri dishes, syringes, receptacles, a camping gas burner, a lighter and a pair of tongs. Field tests have shown that the kit is robust and has been able to stand up to wear and tear even after six months of testing.

Cost estimates for the kit are about US$95 for the eight tests unit and another US$80 for an additional 16 tests unit. The total cost for a 24 test unit would be US$175 or about US$7.3 per test initial cost and a further US$0.44 per test for expendable items such as media and syringes. This cost compares favourably with existing field test kits for coliform bacteria currently available on the market. A survey showed that cost estimates for field kits, such as those made by Millipore and Sartorius, cost in the range of US$1,000-$1,700 for a 20 to 24 tests unit. These costs include an incubator. Per test costs have been estimated to range from US$50 to US$75 initial cost and a further US$0.80 to US$3.00 per test for expendable items.

It is anticipated that costs for the present kit can be brought down if plastic bottles can replace McCartney bottles and pipettes in place of syringes. The most expensive item in the kit is the butane gas burner which costs US$40 and other substitute heaters could possibly be used which are cheaper.

The present kit weights about 6.4 kg and it is possible to reduce this weight if lighter materials, such as plastic bottles in place of glass, can be used. The kit, as it is presently, has not been shown to be difficult to handle, but any reduction in weight would be an advantage if inaccessible areas are being monitored. The kit has been shown to be suited for carriage on a bicycle, which is suitable for monitoring in areas which are not accessible to motor vehicles.

GENERAL CONCLUSIONS

The coliphage test may be viewed as a reliable alternative technique for the assessment of bacteriological water quality, comparable to other standard bacteriological techniques available. It has several advantages in terms of the shorter time taken for the test, and that samples may be stored for longer periods at 4°C and still give reliable estimates of the water quality at the time of sampling. The modified coliphage test adapted into a field kit has been shown to give reliable results and is robust. At least for Malaysian conditions the test can be carried out without the need for an incubator. Cost estimates for the kit and the tests have shown that it is possibly cheaper than those available on the market.

Nevertheless, there is need to improve the field kit further such as in reducing its weight, increasing the sensitivity of the coliphage test, and increasing the number of tests per unit of kit.

ACKNOWLEDGEMENTS

This paper was prepared on behalf of the project team for Malaysia. The study was supported by a grant provided by the International Development Research Centre, Canada, with whom we wish to gratefully acknowledge. We also wish to thank the Ministry of Health Malaysia for their support in testing the kit.

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FIGURE 1

THE LOG_{10} RELATIONSHIP OF COLIFORMS (M-FC) TO COLIPHAGE ENUMERATED IN WELL WATERS

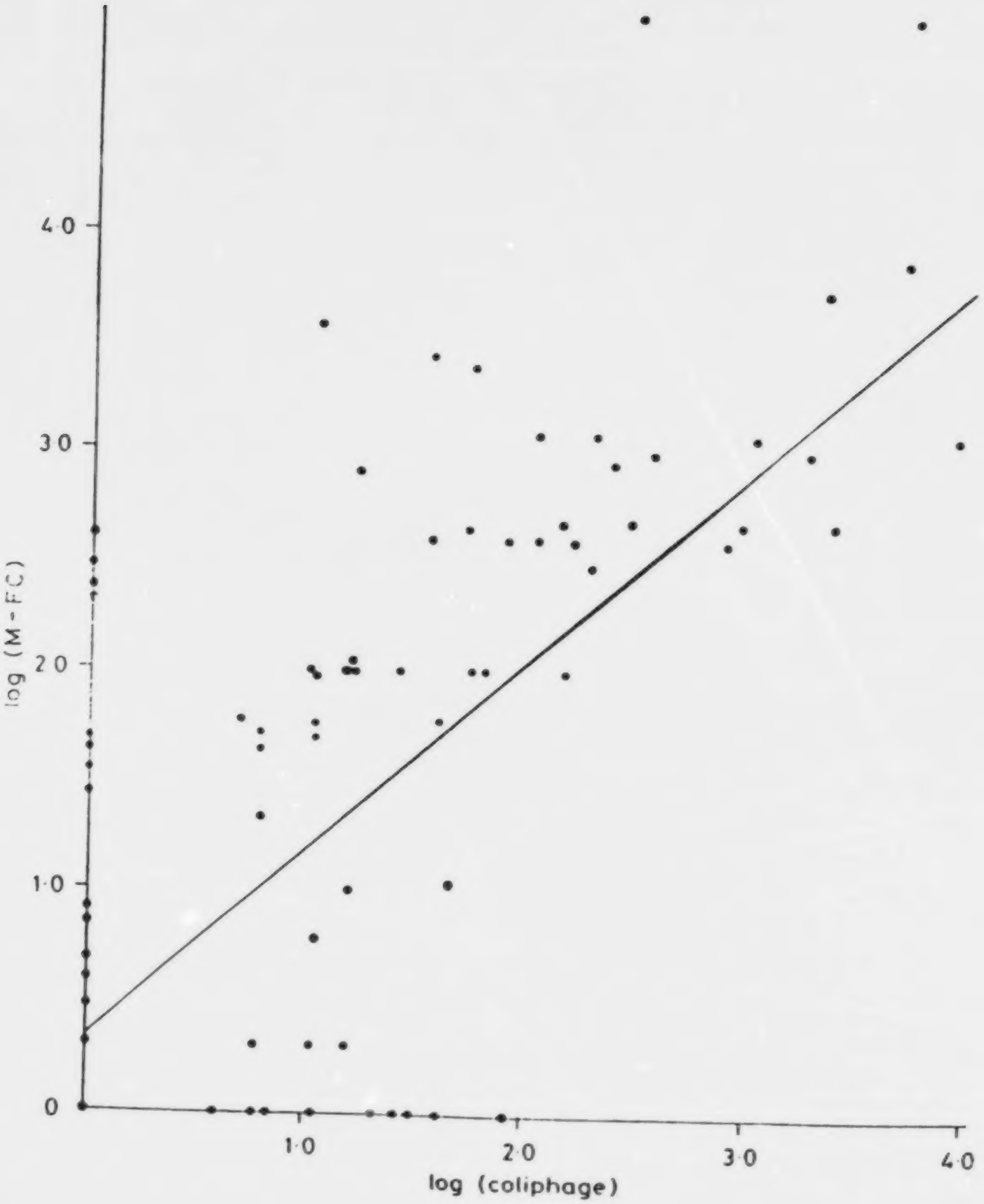


FIGURE 2

THE LOG_{10} RELATIONSHIP OF COLIFORMS (M-FC) TO COLIPHAGE ENUMERATED IN OPEN WELLS, KULIN DISTRICT

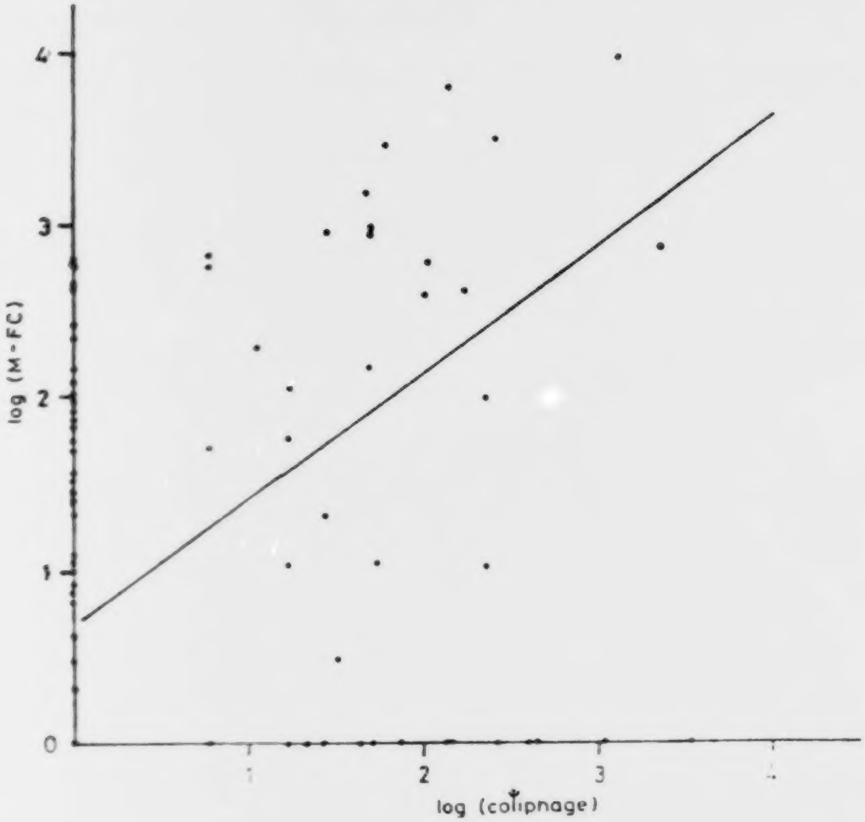
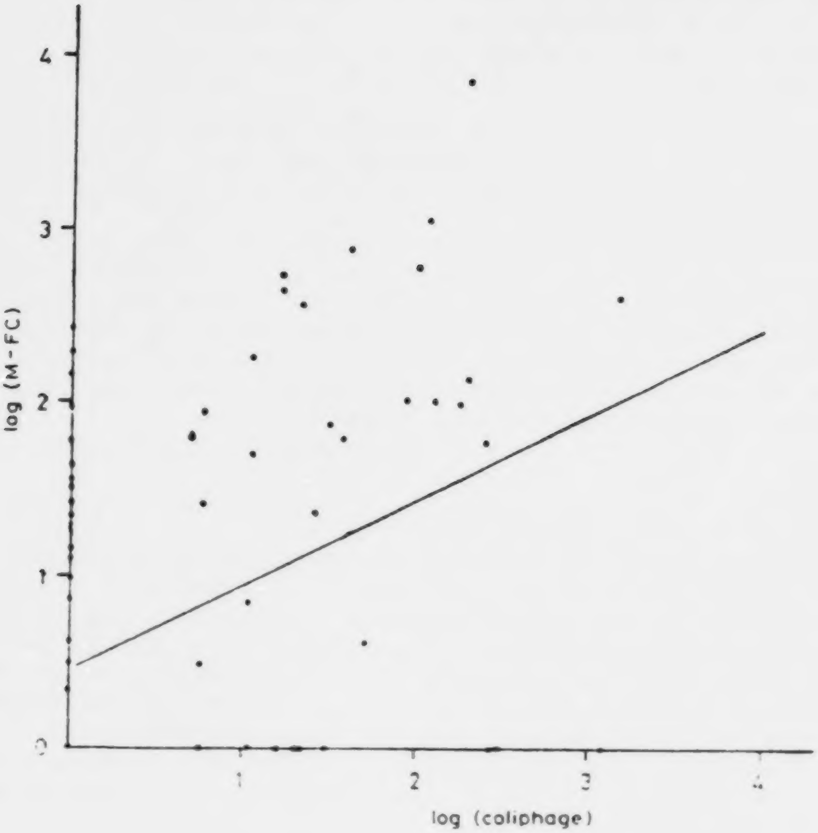


FIGURE 3

THE LOG_{10} RELATIONSHIP OF COLIFORMS (M-FC) TO COLIPHAGE ENUMERATED IN TUBE WELLS, KULIM DISTRICT



AN INTRODUCTION TO THE RAISON SYSTEM

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ABSTRACT

An expert system prototype, originally developed for acid rain problems in Canada, has been adapted for use in water quality problems in Malaysia. The system is acronymed RAISON for regional analysis by intelligent systems on a microcomputer. While some peripheral subsystems required minor modifications, the principal kernel of the computer package remained unchanged during the transition. Test results with preliminary data for hypothetical scenarios demonstrated potential applications of RAISON in the water quality data management in Malaysia, particularly in the development of a prototype classification scheme by using coliphage counts and sanitary survey information.

INTRODUCTION

The Water Quality Data Management Project (Malaysia/Canada) is a cooperative study supported by the International Development Research Centre (IDRC) involving a Malaysian research team (Coordinator: P. Ho; Members: S.L. Tong, C.W. Wang, S.T. Chin, S.T. Teoh, Y.F. Ngeow, C.L. Loh and C.K. Lim) and a Canadian research team (Coordinator: D. Lam; Members: D.A. Swayne, J. Story and A.H. El-Shaarawi). The project is currently halfway into its operation. This report is prepared by the Canadian team to discuss the progress to date. The Malaysian results are given in a separate paper in this book.

The main objective concerning the Canadian component of this cooperative project involves the software development and statistical methods required to develop a prototype classification scheme using coliphage counts and sanitary survey information. The other objective is to enhance and test a computerized water quality data handling and management system for demonstrative and reporting purposes. The system is based on the RAISON computer package developed by the National Water Research Institute and the University of Guelph.

The RAISON system has been developed originally for the regional analysis of the acid rain problems in Canada (Swayne and Fraser, 1986; Lam _et al_, 1988). It utilizes microcomputer technologies such as colour graphics, spreadsheet computations and data files to offer a user-friendly workstation environment for easy data entry and retrieval, manipulation of data files based on spatial maps, simple statistical calculations and backcolouring the results on the maps. It is also used to implement an expert system prototype that can select the appropriate mathematical models by a set of knowledge rules.

The purpose of this report is to present the essential features of the original RAISON system that are relevant for the Malaysian project. To illustrate the use of RAISON, some preliminary data on coliphage counts and other water quality variables are used for testing hypothetical scenarios.

THE RAISON SYSTEM

The RAISON System can be run on an IBM/AT or a compatible with 640K memory, a 20 Mbyte hard disk and an EGA colour monitor and, preferably, a monochrome monitor. It consists of three main components: the map interface, the database system and the spreadsheet facilities. As shown in **Figure 1**, the RAISON/Malaysia project consists of setting up the maps, entering the data and implementing the classification schemes and generating the output, utilizing these three components.

The Map Interface

Maps are entered into the RAISON system by means of digitizing the boundaries of the states, districts and village maps. **Figure 2** shows the national map of Western Malaysia, with the different states. By moving the cursor to a state, a more detailed map of that state can be shown. For example, **Figure 3** shows the districts in the State of Kedah. A pull-down manual allows for addition of new icons, access to spreadsheet data, database, displaying stations, and printing the screen on either a conventional dot matrix printer or a colour printer.

The Database Management System

The data are organized according to sampling stations (e.g. drinking wells) within the district. Data entry and change are possible via the database command in the pull-down manual. **Figure 4(a)** shows the options to create, update, link and import data. In the create mode, the user can specify a layout of his choice by defining the name of the variable, whether numeric or non-numeric, and the length of each record. **Figure 4(b)** shows an example of the data entered for well No. SC01 for data 88/03/16 with information on well types, depth and other sanitary data. **Figure 4(c)** shows data from another well (No. JBS37) on the membrane filter counts (MFC) and the coliphage counts for date 88/04/05. By defining the appropriate layouts and entering the data accordingly or by importing a previously processed file (e.g. from dBASE III files), one can easily install various data files in RAISON.

One of the special features in RAISON is the ability to retrieve from any of these data files those variables that are of interest. For example, as shown in **Figure 5(a)**, one can use the cursor to draw a polygon around those stations in the village of Semenyih (in the District of Ulu Langat in the State of Selangor) that are of interest to the user and use the polygon command to retrieve the MFC and coliphage data for further analysis on the spreadsheet (**Figure 5(b)**).

The Spreadsheet

The spreadsheet in RAISON is designed to appear familiar to someone who has used the commercial variety. The options are available at the top of the spreadsheet, to access data files and worksheets, to calculate statistical means and medians or to compute according to a preset formula, to backcolour stations or regions according to some user-specified functions.

While these simple mathematical operations provide fast and convenient results, more complex computations are best handled by programming. A new language, the RAISON Programming Language (RPL), is developed for this purpose. This language is written originally in the C Language and is made for easy use by programmers familiar with the BASIC Language. The program can be stored in a file and accessible in the spreadsheet by the command, "runprog". It can also be run separately outside the spreadsheet. Typically, the RPL programs handle repetitive computations for a large number of data files, e.g. for all stations in a district, state or even the whole nation. It has, however, the disadvantage of any interpreter language, namely slow operations. The alternative is to write the program in the C Language. Experiences with the acid rain application indicated that the RPL program could be five to ten times slower than the corresponding C program. Depending on the type of application, e.g. the classification scheme for the water quality data, one can use either language in RAISON. In the more advanced applications such as non-numeric information processing, LISP or Prologue (**Figure 1**) are preferred but probably not required for this project.

RESULTS

Since this project has only reached its halfway point, the final results are not yet available. The data handling and management systems are already operational, e.g. data entry and retrieval through the different levels of map and the use of spreadsheets and RPL programs. **Figure 2** presents the information on the number of houses for each state by using the method of backcolouring. The colours shown are based on a formula specified in the spreadsheet in which the original data were retrieved. **Figure** 3 presents the results of using plotting features in RAISON for line and bar graphs. It shows the time series data for hypothetical cases of typhoid (**Figure 3(a)**) and infectious hepatitis (**Figure 3(b)**) in some selected districts in the state of Kedah. These figures would be helpful for analyzing the time trends and spatial distributions of these community and health data. For example, the yearly averaged number of typhoid cases in each district can be computed with the "AVG" function available in the spreadsheet. A colouring function can then be defined to segregate these average values into classes. The result is presented in **Figure 6(a)**, using the backcolouring option in the spreadsheet (**Figure 6(b)**).

The analysis of different types of data is made possible by retrieving the appropriate data from the various data files. For example, the time series data of population, coliphage and MFC can be retrieved and displayed on the screen (**Figure** 7(**a**)) alongside the map of the state of Kedah. Backcolouring is used to highlight the district (Kuala Mula) from which the water quality and sanitary survey data were collected. By moving the cursor to another district (Baling), similar data can be retrieved and displayed (**Figure** 7(**b**)). Thus, one can browse through all the districts quite quickly and conveniently. One can also derive the linear regression relationship between any two variables (e.g. MFC vs. Coliphage) from the data and plot the regression line alongside the district (**Figures** 7(**a**) and (**b**)). These graphical features can be applied to all levels of maps (i.e. states, districts or villages), as long as the data files are compatible. These simple statistical plots are useful in the development of the classification scheme required for this project.

DISCUSSION

The RAISON system has been adapted to the water quality data management application in Malaysia with minor modification of the original system. In particular, several of the screen graphics facilities were improved to permit simultaneous display of time series data. Preliminary tests showed that the system met the requirements of the new data environment satisfactorily. Without an intensive training session and relying only on user manuals and correspondences, we were able to transfer the RAISON/Malaysia system to the Malaysian team for their use (i.e. data entry, graphics handling and writing RPL programs) in the first three to four months of this project.

In the sense of being portable from one problem (acid rain) to another (water quality) and from one country (Canada) to another (Malaysia), the RAISON expert system prototype has partially demonstrated its use as a so-called "shell". Further tests such as the manipulation of non-numeric data and the use of decision rules in the development of the classification scheme are needed to establish its versatility.

ACKNOWLEDGEMENTS

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FIGURE 1

COMPONENTS IN RAISON/MALAYSIA:
MAPS, STATISTICS AND DATA

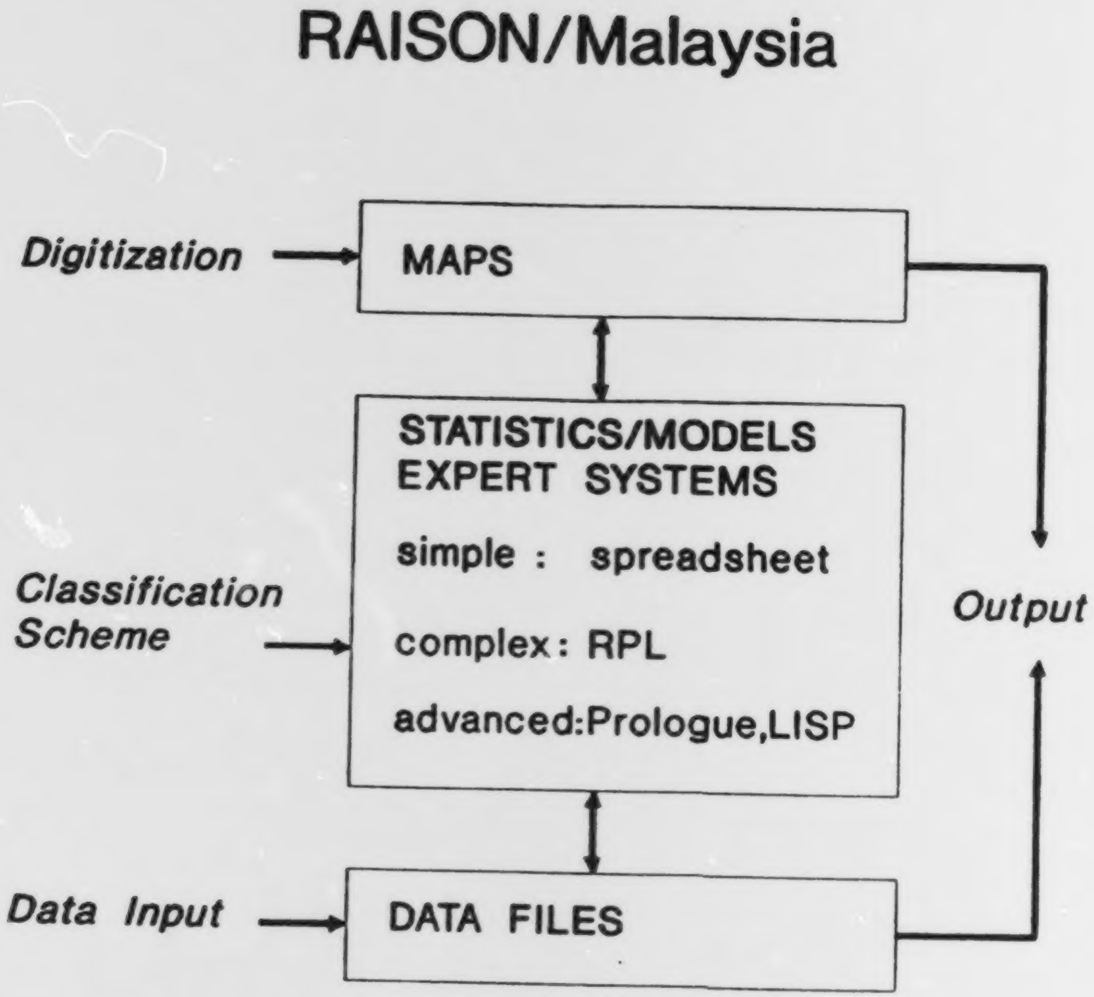
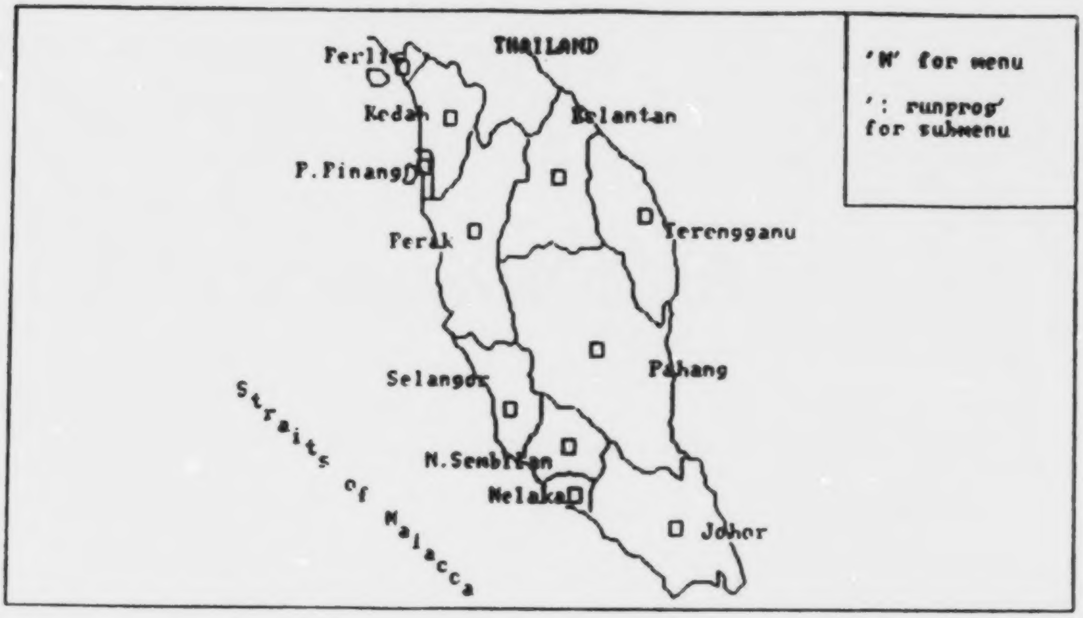


FIGURE 2

WESTERN MALAYSIA: THE STATES AND THE NUMBER OF HOUSES



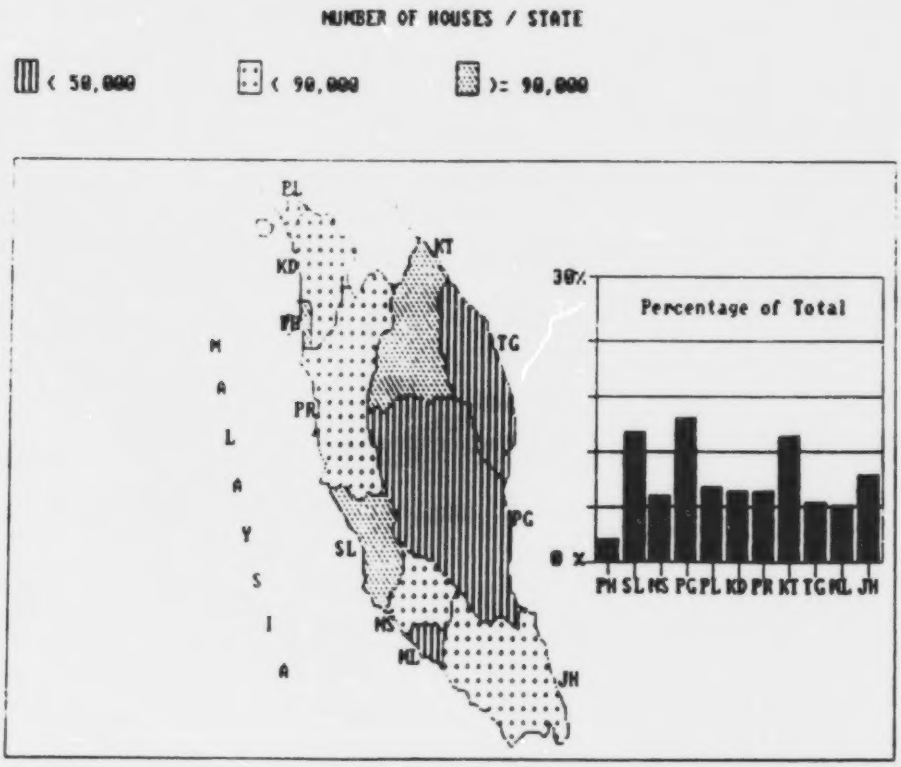
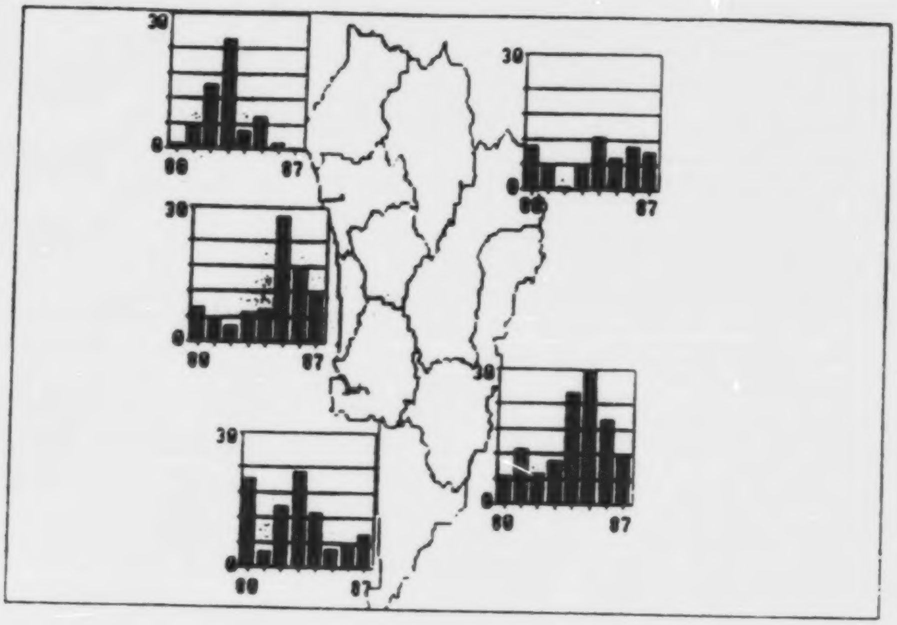


FIGURE 3

TIME SERIES HEALTH DATA FOR THE KEDAH STATE: (A) TYPHOID AND (B) HEPATITIS CASES, 1980-1987

(a)

TYPHOID CASES IN KEDAH
(hypothetical)
FROM 1980 TO 1987



(b)

INFECTIOUS HEPATITIS CASES IN KEDAH
(hypothetical)
FROM 1980 TO 1987

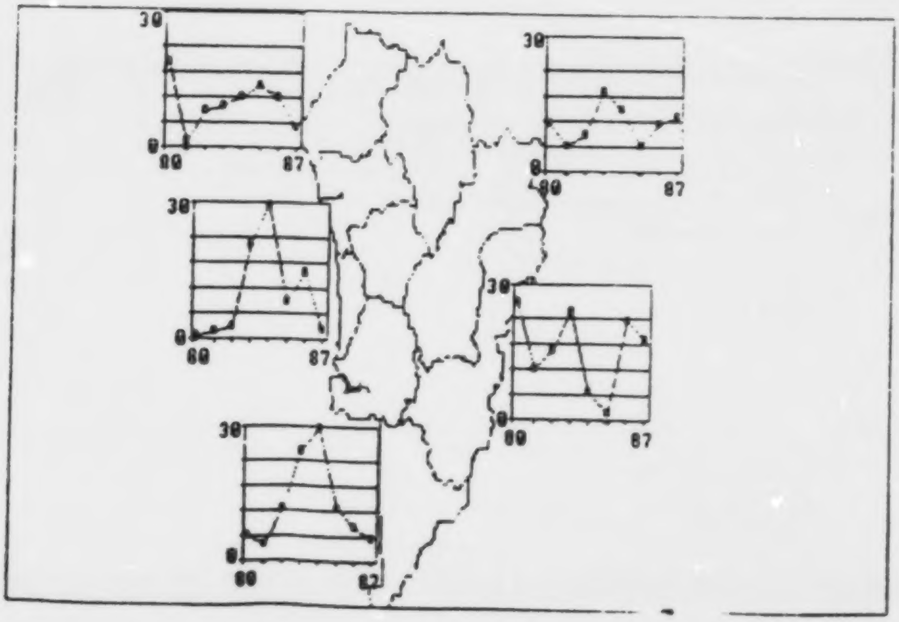
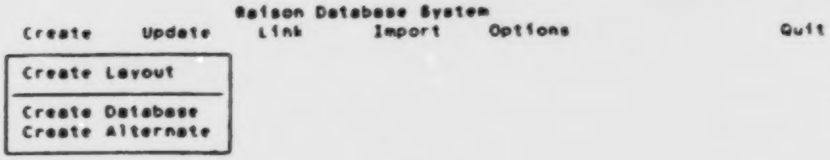


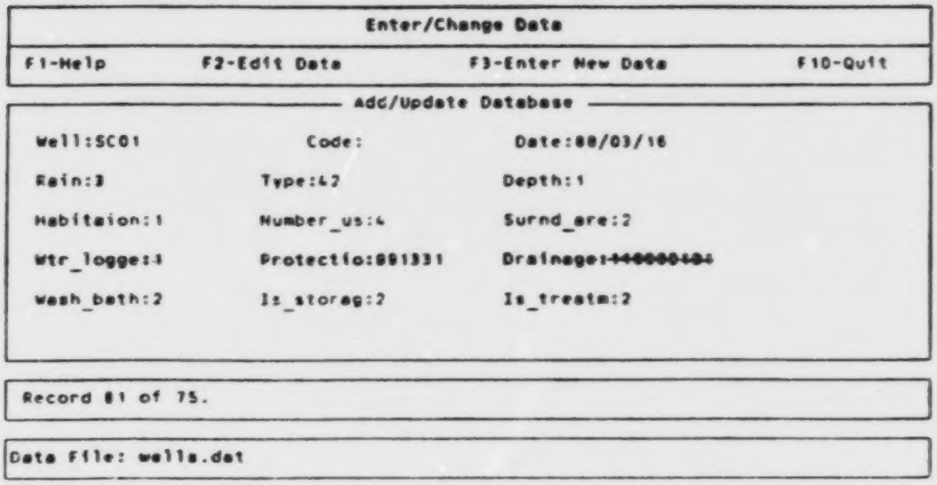
FIGURE 4

(A) RAISON DATABASE SYSTEM; (B) SANITARY DATA OF WELL NO. SC01 IN THE WELLS.DAT FILE; (C) WATER QUALITY DATA OF WELL NO. JB837 IN THE SEMENYIH.WQD FILE

(a)



(b)

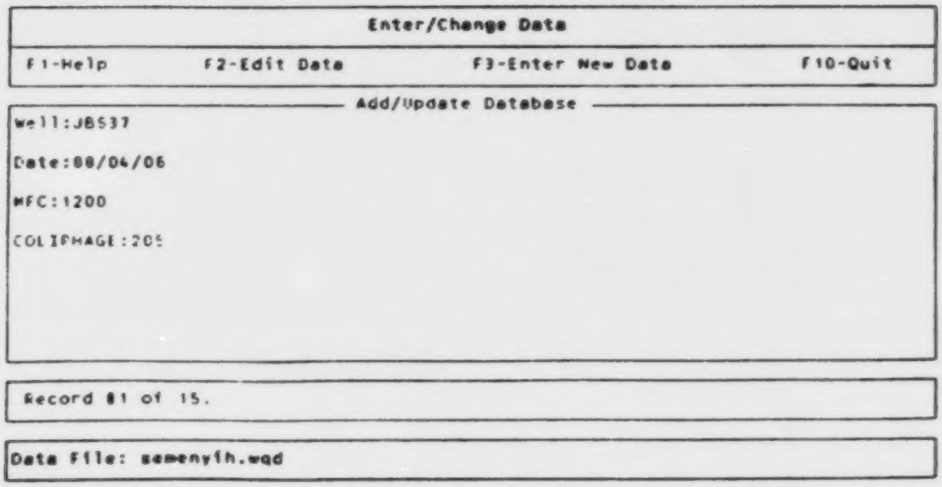


Well:SC01
Code:
Date:88/03/16
Rain:3
Type:42
Depth:1
Habitaion:1
Number_us:4
Surnd_are:2
Wtr_logge:1
Protectio:881331
Drainage:[illegible]
Wash_bath:2
Is_storag:2
Is_treatm:2

Record #1 of 75.

Data File: wells.dat

(c)



Well:JB537
Date:88/04/06
MFC:1200
COLIPHAGE:205

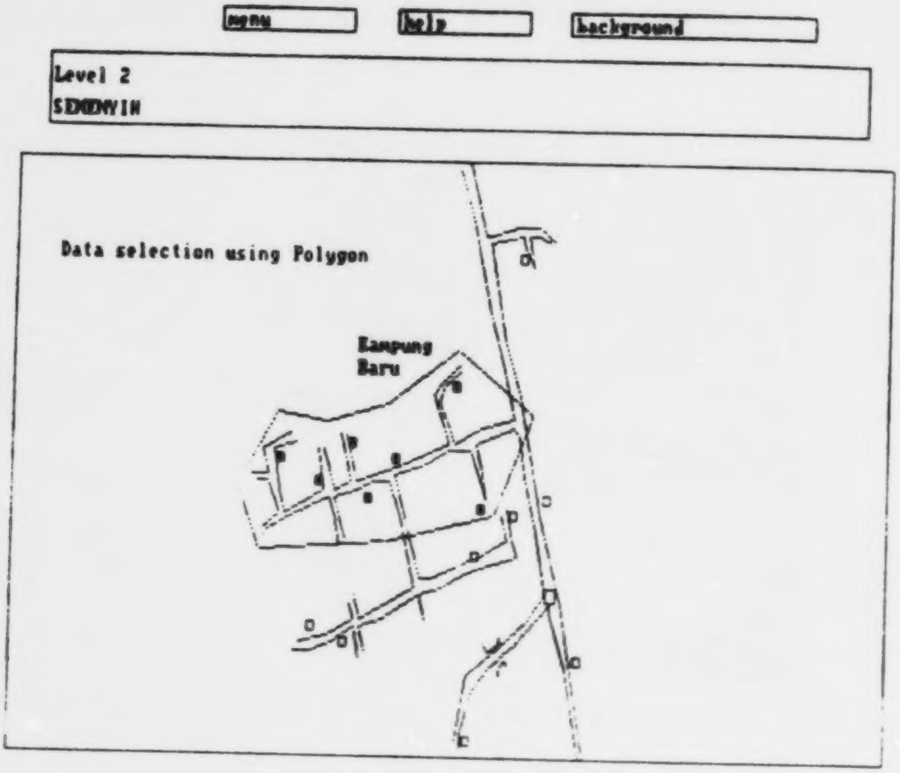
Record #1 of 15.

Data File: semenyih.wqd

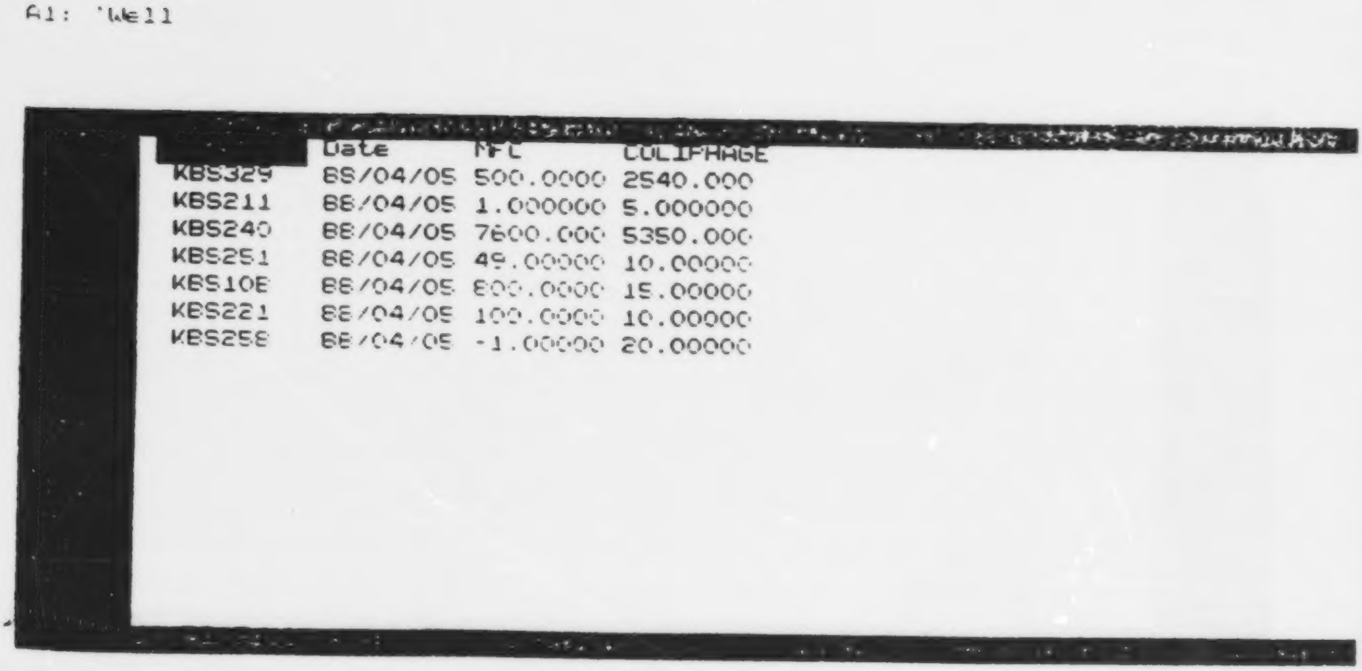
FIGURE 5

(A) RETRIEVAL OF DATA WITH THE "POLYGON" COMMAND;
(B) SPREADSHEET SHOWING THE DATA WITHIN THE POLYGON

(a)



(b)



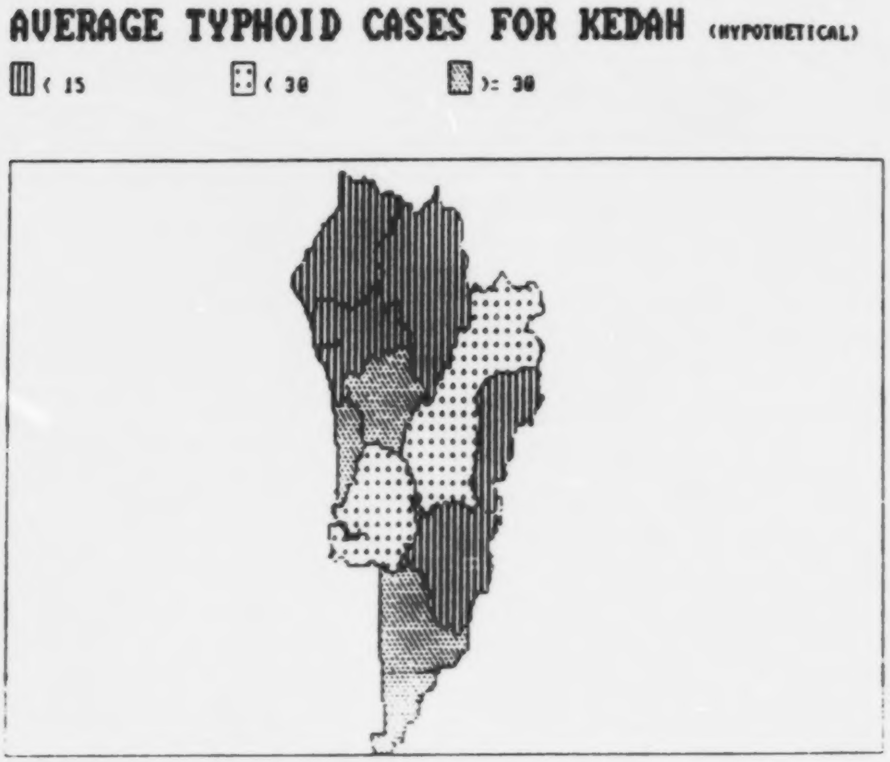
```
A1: 'Well

          Date     MFL      COLIPHAGE
KBS329    88/04/05 500.0000 2540.000
KBS211    88/04/05 1.000000 5.000000
KBS240    88/04/05 7600.000 5350.000
KBS251    88/04/05 49.00000 10.00000
KBS108    88/04/05 800.0000 15.00000
KBS221    88/04/05 100.0000 10.00000
KBS258    88/04/05 -1.00000 20.00000
```

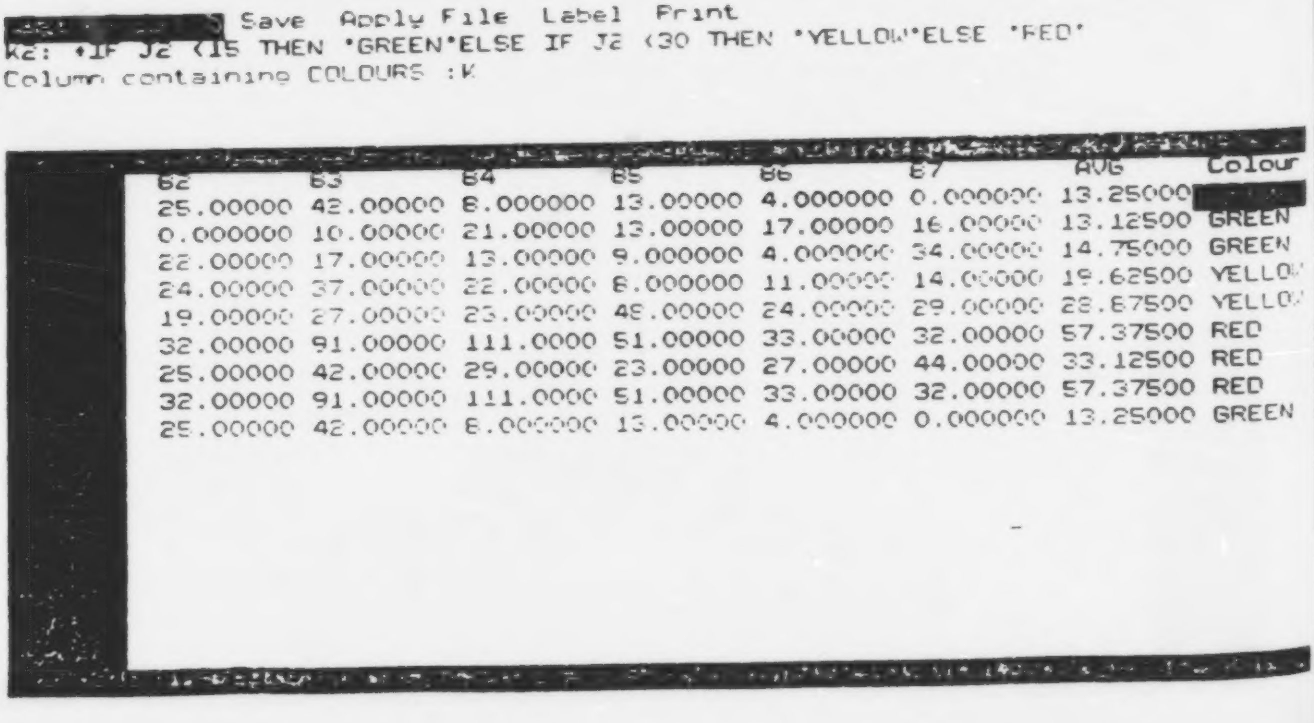
FIGURE 6

YEARLY AVERAGE TYPHOID CASES IN KEDAH AND THE RELATED SPREADSHEET COMPUTATIONS

(a)



(b)



```
[illegible] Save  Apply File  Label  Print
K2: +IF J2 <15 THEN 'GREEN'ELSE IF J2 <30 THEN 'YELLOW'ELSE 'RED'
Column containing COLOURS :K

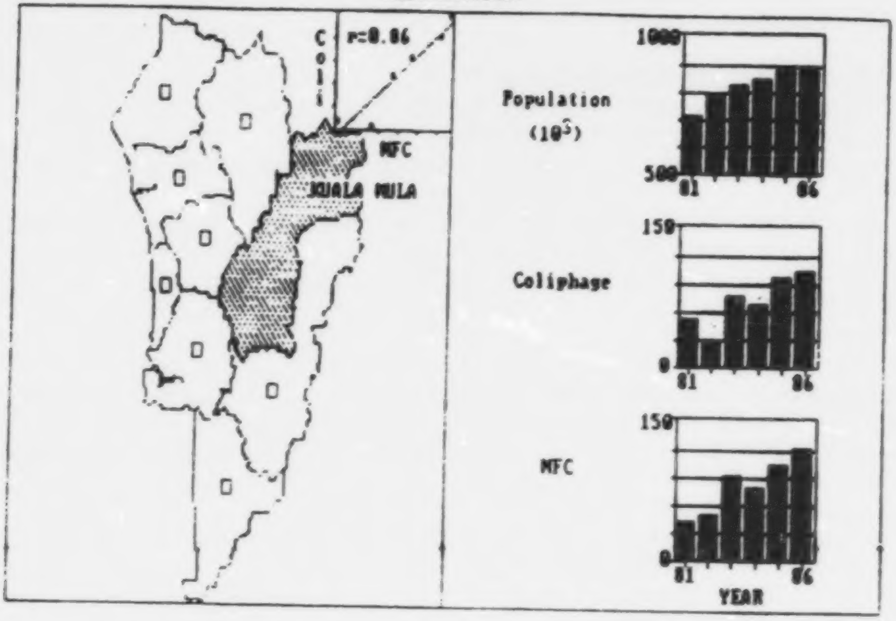
 82       83       84       85       86       87       AVG      Colour
 25.00000 42.00000 8.000000 13.00000 4.000000 0.000000 13.25000 [illegible]
 0.000000 10.00000 21.00000 13.00000 17.00000 16.00000 13.12500 GREEN
 22.00000 17.00000 13.00000 9.000000 4.000000 34.00000 14.75000 GREEN
 24.00000 37.00000 22.00000 8.000000 11.00000 14.00000 19.62500 YELLOW
 19.00000 27.00000 23.00000 48.00000 24.00000 29.00000 28.87500 YELLOW
 32.00000 91.00000 111.0000 51.00000 33.00000 32.00000 57.37500 RED
 25.00000 42.00000 29.00000 23.00000 27.00000 44.00000 33.12500 RED
 32.00000 91.00000 111.0000 51.00000 33.00000 32.00000 57.37500 RED
 25.00000 42.00000 8.000000 13.00000 4.000000 0.000000 13.25000 GREEN
```

FIGURE 7

TIME SERIES DATA OF POPULATION, COLIPHAGE AND MFC FOR
(A) KUALA MULA DISTRICT AND
(B) BALING DISTRICT IN THE STATE OF KEDAH WITH
LINEAR REGRESSION RESULTS

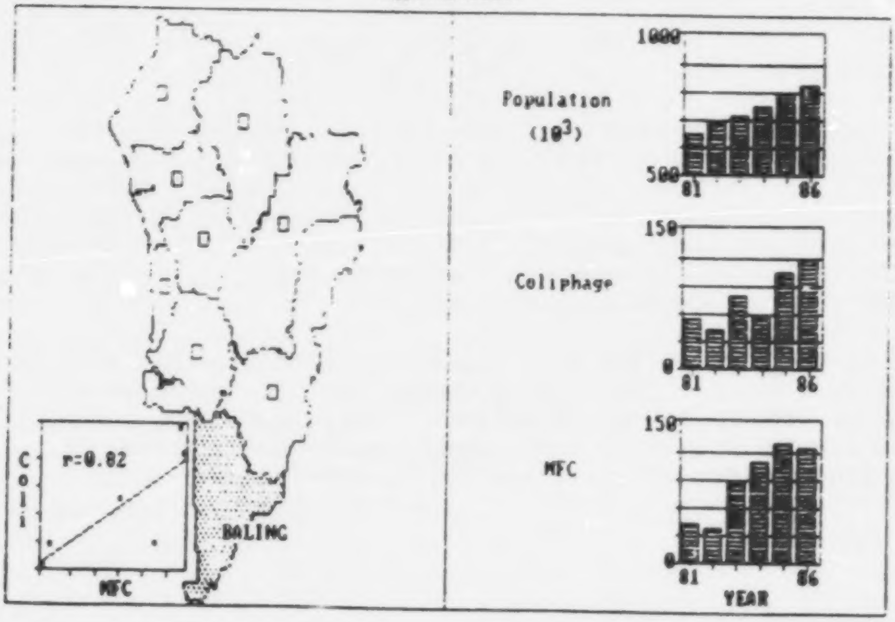
KUALA MULA, KEDAH
1981 to 1986
(hypothetical)

(a)



BALING, KEDAH
1981 to 1986
(hypothetical)

(b)



RAISON-MALAYSIA: A USER-FRIENDLY SOFTWARE PACKAGE FOR WATER QUALITY DATA MANAGEMENT (MALAYSIA/CANADA)

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ABSTRACT

Recent progress in the development of a user-friendly software package, RAISON-Malaysia for water quality data management, is reported in this paper. RAISON-Malaysia was developed using the software package RAISON which incorporated a map system, a database and a spreadsheet package, as the basic framework. The system design of RAISON-Malaysia was based on the water quality management requirements identified with specific reference to the rural water supply, sanitation, and drinking water quality surveillance programme in Malaysia as a case model. The system is basically a multi-level, menu-driven geographical information system. Sample outputs at the various levels are shown to illustrate the built-in facilities and capabilities of the system for water quality management.

INTRODUCTION

This paper forms part of an interim report of the cooperative project sponsored by the International Development Research Centre (IDRC) of Canada on Water Quality Data Management (WQDM) (Malaysia/Canada) involving the University of Malaya (UM) and the Department of Environment (DOE) of Malaysia on the one part, and the National Water Research Institute (NWRI) and the University of Guelph (UG) of Canada on the other.

The main objective of this project is to develop and test a user-friendly computer software package for the management and analysis of data related to drinking water quality, incorporating a classification system for categorizing rural water supply sources according to "relative risk" to the consumer.

The specific objectives of this project are:

1. To gather and examine available water quality data and identify input and output requirements in collaboration with the Ministry of Health;

2. To undertake a sanitary survey of the different water supply sources and identify those common variables that affect bacteriological water quality;

3. To develop a prototype classification scheme for determining the suitability of water supply sources for human consumption by using coliphage counts and the results of sanitary surveys as the principal quality indicators;

4. To develop, enhance and test a computerized water quality data handling and management system (user-friendly software package) for use on microcomputers, based on the RAISON system developed by NWRI in collaboration with UG:

   (a) to adapt the RAISON system to meet the data handling requirements of Malaysia as identified in objective 1,

   (b) to assess its potential to store and analyze water quality data including the design of an input format,

   (c) to produce enhancements such as redesign of the database management routines, improved data entry facilities and the source classification scheme developed in 3,

   (d) to develop (for demonstration purposes) a series of reports and relevant statistics for use by those agencies responsible for water quality surveillance.

The project tasks within the Malaysian component were divided between two task groups. Task group one was responsible for the collection of bacteriological data on water samples and carrying out sanitary surveys of selected rural water sources. The second task group focused on the development of a water quality data management software and classification scheme for sources of water supply.

The Ministry of Health of Malaysia is involved as a collaborating agency in identifying the needs and testing of tne water quality data management system. The Ministry and the state Departments of Health also have been providing assistance in field sampling, testing and the sanitary survey.

To date, field testing of about 600 well water sources has been completed. The testing included measurements of coliphage counts and faecal coliforms (membrane procedure) and was accompanied, for most water sources, with a sanitary survey. These data provided the input to the second part of the study. This report will focus on the development of the user-friendly water quality data management system as well as statistical analyses being studied for the classification of water sources.

DEVELOPMENT OF THE RAISON-MALAYSIA WQDM SYSTEM

Hardware Requirements

The microcomputer system set-up for the present study consists of an ACER 910 80286-based microcomputer equipped with 1 MB RAM, a 1.2 MB and a 360 kB floppy disk, a 40 MB hard disk, a monochrome and a high resolution colour monitor, a Logitec K-510 digitizer, and an HP-Inkjet colour plotter/printer. A lower cost IBM PC-XT compatible with single or dual monitors also can be used with most of the useful functions of the WQDM package available, except that the colour display may not be as good.

The RAISON Package

The software package RAISON developed by NWRI/UG for the analysis of data on acid rain has been adapted as a framework for the development of the RAISON-Malaysia Water Quality Data Management system. The original RAISON is an integrated package incorporating a database management system, a mapping package, a spreadsheet, and a statistical program entitled "Epistat". The original features available and the new features added by the NWRI/UG group in response to the needs of the WQDM project in the RAISON package have been discussed in the previous paper (Lam *et al*).

The RAISON-Malaysia WQDM System Design

The system design of the RAISON-Malaysia package was based on the general requirements envisaged in the water quality management by governmental agencies involved in water supply and sanitation in rural areas. The needs by the Ministry of Health on water quality management for rural Malaysia have been considered in this study as a case model. References were made to a report on the status of water supply and sanitation in rural areas of one of the states in West Malaysia prepared by the Division of Engineering Services, Ministry of Health, Malaysia (1987). The report describes the existing water supply and sanitation situation in rural areas in the state, and in addition, provides general background information such as physical and climatic conditions, population, public health conditions in terms of the statistical occurrence of different types of water-borne diseases and other information. The statistics and information presented in the report in the form of tables, figures and maps were shown in relation to geographical distribution and over a period of several years. The report served as a background paper for the purpose of development planning for the Ministry at both the state and federal levels.

Also considered in determining the needs in water quality management in rural water supply was the "Manual on Drinking Water Quality Surveillance" (Ministry of Health, Malaysia, 1983). The surveillance programme of the Ministry of Health of Malaysia includes water quality testing and sanitary surveys. The implementation of the monitoring programme, the storage and analysis of the water quality data and sanitary information, and the necessary follow-up and remedial actions have not been as efficient as would have been desired. Computerized management is currently being considered by the Ministry.

RAISON-Malaysia was designed with an attempt to meet most of these requirements in WQDM encountered in the case study model described above. Within the framework of the RAISON package, a multi-level and menu-driven geographical information system (GIS) has been developed which is interactive with the database and spreadsheet subsystems within the RAISON package. The database provides the links between the map files in vector graphics (national, state and district maps and maps of water sources in villages) and the water quality data or other statistical information. The RAISON database can import data easily from dBase III files. Large water quality data or other relevant files were stored in dBase III for its versatility in data reduction. The GIS map presentations of water quality data and other relevant statistical information were achieved using the "BACKCOLOUR" function in the RAISON spreadsheet. The GIS programmes were written within the RAISON environment and the interactions with the database and spreadsheet are transparent to the users. An overall system flow diagram will be described in a later section.

The R-M package was targeted for two levels of users. It may be operated by people with little or no previous exposure to microcomputers, such as health officers in rural areas. For this purpose, most of the functions were, as far as possible, executable through single key-strokes. For users who are responsible for water quality management at the state or national level, there will be easy accessibility to the combined databases from different regions within the state or several states. Flexibility will be built in to allow them to add or change functions in data analysis, and in map presentation to meet their needs in management and planning.

Map Digitization

State maps of the eleven states in Peninsular Malaysia showing details of district boundaries have been digitized in sections using a Logitec K-510 digitizer and the "Crosstalk" programme to obtain primary digitized files. These files consist of many sections made up of line segments. Common boundaries were digitized only once as a common line segment to avoid any discrepancies when different sections of the files were combined. Accurate scale maps were used and conversion of digitizer pair-coordinate outputs to longitude-latitude points were carefully calibrated with the aid of a simple built-in programme. The digitized files were combined to form the national map (for Peninsular Malaysia). The map display can be zoomed in to provide resolution as low as approximately 0.5 km.

Water quality monitoring normally involves sampling of water sources in relatively close proximity in scatter regions, such as villages. In the present map system, village locations were identifiable in the digitized maps at higher levels. However, the display of the water sources/sampling stations required a ten-fold expansion in the longitude and latitude scales. These station map files were independent from the higher level maps and the only linkage was via the common village locations.

A procedures manual is being compiled to allow users to adopt a systematic approach in the digitization of the higher and lower level maps for use in the GIS.

Data Input

To date about 430 field testing data on bacteriological quality of about 400 water sources (wells) are available in terms of coliphage and membrane faecal coliform (MFC) counts. These have been stored in dBase III files and imported into the R-M system database and utilized for the testing, redesign in display, and programming within the R-M system environment. The village map files of the first batch of about 230 data points covering stations in 9 regions (villages), 4 each from Selangor and Negeri Sembilan, and 1 from Pahang, have been completed. The latter batch of 200 data points is awaiting sketch location maps of the sampling stations to be digitized into map files.

Sanitary survey records for the corresponding sampling stations were available in the original format designed for field survey. A new database file has been designed for the storage of the survey records in computerized database. The database file constitutes 4 sections, namely: (a) type of water supply; (b) sanitary protection; (c) polluting sources; and (d) land use (see Appendix). Numerical values were assigned to answers in the questionnaire, such as 1 for "yes", 2 for "no", 8 for "do not know", 9 for "no answer", etc. This design was to facilitate later retrieval and statistical analysis for the purpose of source classification.

RESULTS

The R-M Geographical Information System

Figure 1 shows a flow diagram of the R-M Water Quality Data Management System where the current built-in facilities and capabilities are indicated. Most of the options are now operational except the statistical analysis section which represents the proposed approaches currently under testing.

The starting of the system will provide options to access the geographical information system (GIS) directly, or through the main menu to select database, spreadsheet, or statistical analysis operations. The GIS is a multi-level and menu-driven system. Sample outputs will be given below to illustrate each of the functions shown.

Level 0 - National

The entry level of the GIS (**Figure 2**) allows the display of the relevant information at the national level. The addition of the programmes written within the R-M environment has made it possible to have more user-friendly and easy-to-use features incorporated. The available options are shown in the submenu (**Figure 1**). **Figures 3, 4 and 5** show typical displays of statistical information on population and well distribution which were retrieved automatically from the database. Similar displays of statistics on water-borne disease occurrence, health services provision, etc., can be incorporated. The "COLOUR STATES" function can be used to classify, for example, the relative frequency of any of the above-mentioned statistical information on maps following a chosen colour scheme.

The programmes developed also allowed labelling with text in any part of the graphic screen.

Level 1A - State

The functions are similar as in level 0, but information is accessed on state basis. Typical displays are shown in **Figures 6, 7, 8 and 9**.

Level 1B - District

The submenu for this level in **Figure 1** shows that all the facilities for level 0 and level 1A were available here. In addition, two more functions were introduced to allow users to add a new sampling region if necessary, and to incorporate a digitized village station icon file to the corresponding district file. **Figure 10** shows a typical district map with 4 sampling regions. **Figure 11** shows a typical digitized map of sampling stations (wells).

Level 2 - Village Water Quality Display

This is the level where individual water supply sources are shown on the map display. Three basic functions are available and described as follows:

> "COLOUR STATION" - Via the Submenu "Level 2.1", this allows classification of the water quality of water sources (stations) using appropriate colour scheme, e.g. class I - blue; class II - green; class III - yellow; class IV - bright red; and class V - dark red. Different shades were used for monochrome display and black/white print-outs. The classification can be based on: (i) water quality in terms of MFC counts; (ii) water quality in terms of coliphage counts; (iii) ranking according to physical test results; (iv) sanitary condition ranking; and (v) overall water quality ranking. The classification scheme will be based on the statistical models derived using the "STATISTICAL ANALYSIS" programmes. Preliminary results of a simple classification scheme for the water sources of a few sampling regions based on coliphage counts and MFC data are shown in **Figures 12 to 21**. The relevant data were extracted through the built-in program from the R-M databases into the spreadsheet; and the map display of the water quality is by means of the "BACK-COLOUR" function in the spreadsheet. These processes are essentially transparent to the users. Data used and classes assigned to the water sources can be examined readily in the spreadsheet (an example is shown in **Figure 22**). The map files on water quality classification can be saved for quick browsing.
>
> "BROWSING WQ MAPS" - Via submenu "Level 2.2", this allows rapid scanning of time-series water quality map displays, in terms of coliphage counts (**Figures 23 to 26**), MFC counts, physical test ranking, sanitary conditions, or overall water quality ranking.
>
> "ADD STATIONS TO MAP" - Via submenu "Level 2.3", this allows easy updating of new stations in map files.

The R-M Database and Spreadsheet Subsystems

The R-M database subsystem provides a few basic essential functions such as "CREATE", "UPDATE", "LINK" and "IMPORT". Currently five basic types of R-M database files have been created and these are listed in **Figure 1**.

The system permits importation of data files from dBase III. Primary water quality data and sanitary survey records were input into dBase III files. This was in view of the versatility of dBase III package especially where complex data analyses may be required.

The R-M spreadsheet provides the interactions for the display of database information on map files via the "BACKCOLOUR" function. Other functions common in other spreadsheets such as "RECALC", "RANGE", "RETRIEVE", etc., are also available. The "GRAPH" function has now been upgraded by the NWRI/UG group as described in Dr. Lam's paper. The improvements introduced in response to the suggestions of the UM group have greatly enhanced the capability for the graphic presentation of data in overlay of the maps. This is complementary to the built-in functions for graphic overlay to the maps in the R-M GIS system described in the previous section.

Mathematical Model Based on Statistical Analysis and Risk Assessment

Three classes of factors are listed in **Figure 2** under "STATISTICAL ANALYSIS" to be considered in deriving the source classification scheme. The model for the classification will be based on the results of microbiological analysis and physical tests of water samples, and information from sanitary surveys, and hence relative risks to public health. Work is now in progress in the development of the statistical models.

ACKNOWLEDGEMENTS

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Ministry of Health, Malaysia. 1983. Manual on Drinking Water Quality Surveillance (Division of Engineering Services).

Ministry of Health, Malaysia. 1987. Report on the Status of Water Supply and Sanitation in the Rural Areas of Kedah (Division of Engineering Services).

Presented at "IDRC End-of-Project Meeting, Water Quality Control Network", Banff, Alberta, September 4-8, 1988.

FIGURE 1 (page 1 of 2)

RAISON-MALAYSIA SYSTEM FLOW DIAGRAM

RAISON-MALAYSIAN

WATER QUALITY DATA MANAGEMENT SYSTEM

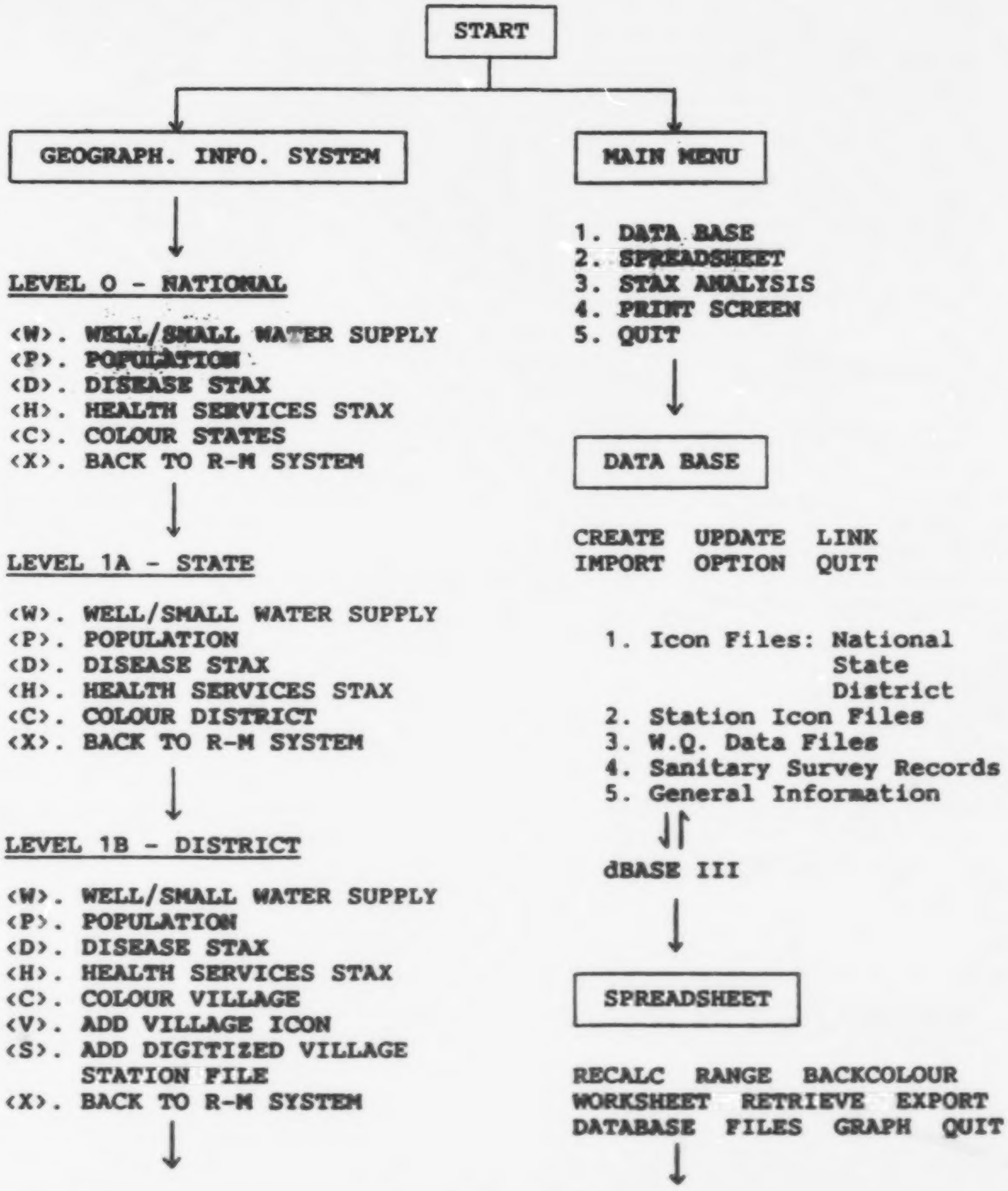


FIGURE 1 (page 2 of 2)

RAISON-MALAYSIA SYSTEM FLOW DIAGRAM

↓

LEVEL 2 - VILLAGE WATER QUALITY DISPLAY

<C>. COLOUR STATION
<B>. BROWSING WQ MAPS SAVED
<A>. ADD STATION TO MAP
<X>. BACK TO R-M SYSTEM

↓

LEVEL 2.1 - COLOUR STATION

<R>. RETRIEVE DATA FROM R-M DATABASE & CLASSIFICATION
<D>. DISPLAY DATA IN SP_SHEET
<G>. GO - COLOUR THE STATION
<S>. SAVE (spreadsheet & map)
<X>. EXIT SUBMENU

↓

LEVEL 2.2 - BROWSING

<M>. MFC WQ MAP
<C>. COLIPHAGE WQ MAP
<P>. PHYSICAL TEST RANKING
<S>. SANITARY CONDITION
<R>. WQ RANKING MODEL
<D>. DISPLAY SPREADSHEET
<PgUp>. PAGE UP
<PgDn>. PAGE DOWN
<X>. EXIT BROWSING

↓

LEVEL 2.3 - ADD STATION TO MAP

Crosshair movement
-- 4 arrow keys, <Home>, <End>, <PgUp>, <PgDn>
-- Speed keys <+>, <->

<enter> -- add station locations to file
<i> -- identify location
<esc> -- exit
<h> -- help

↓

STATISTICAL ANALYSIS

1. Mathematical model for classification based on coliphage/MFC data.

   Negative binomial distribution fitting.

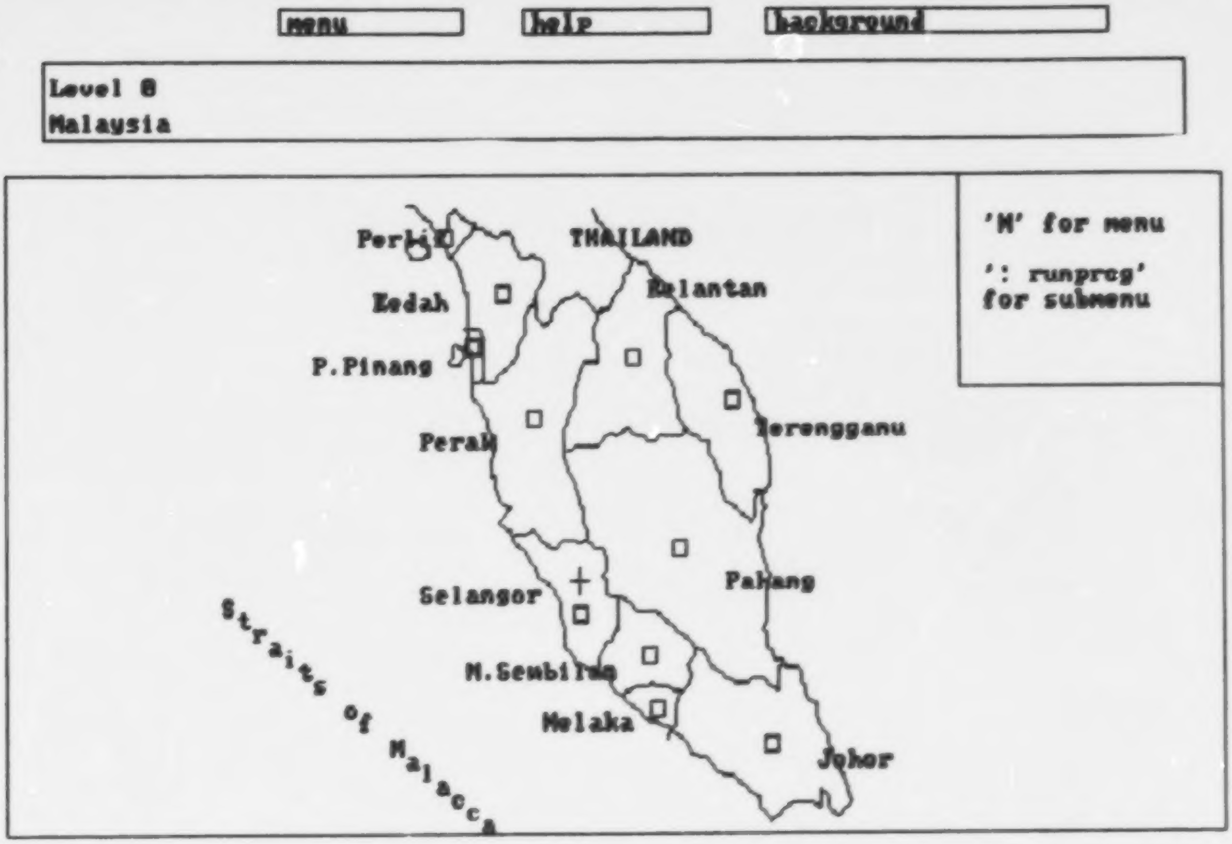
   Classification into groups - use index of dispersion, risk assessment.

2. Sanitary survey records- correlational analysis with coliphage counts & MFC.

   Develop index based on risk assessment.

3. Other water quality factors, physical tests: turbidity, colour, odour, taste.

4. Overall W.Q. index - Mathematical model based on risk assessment.

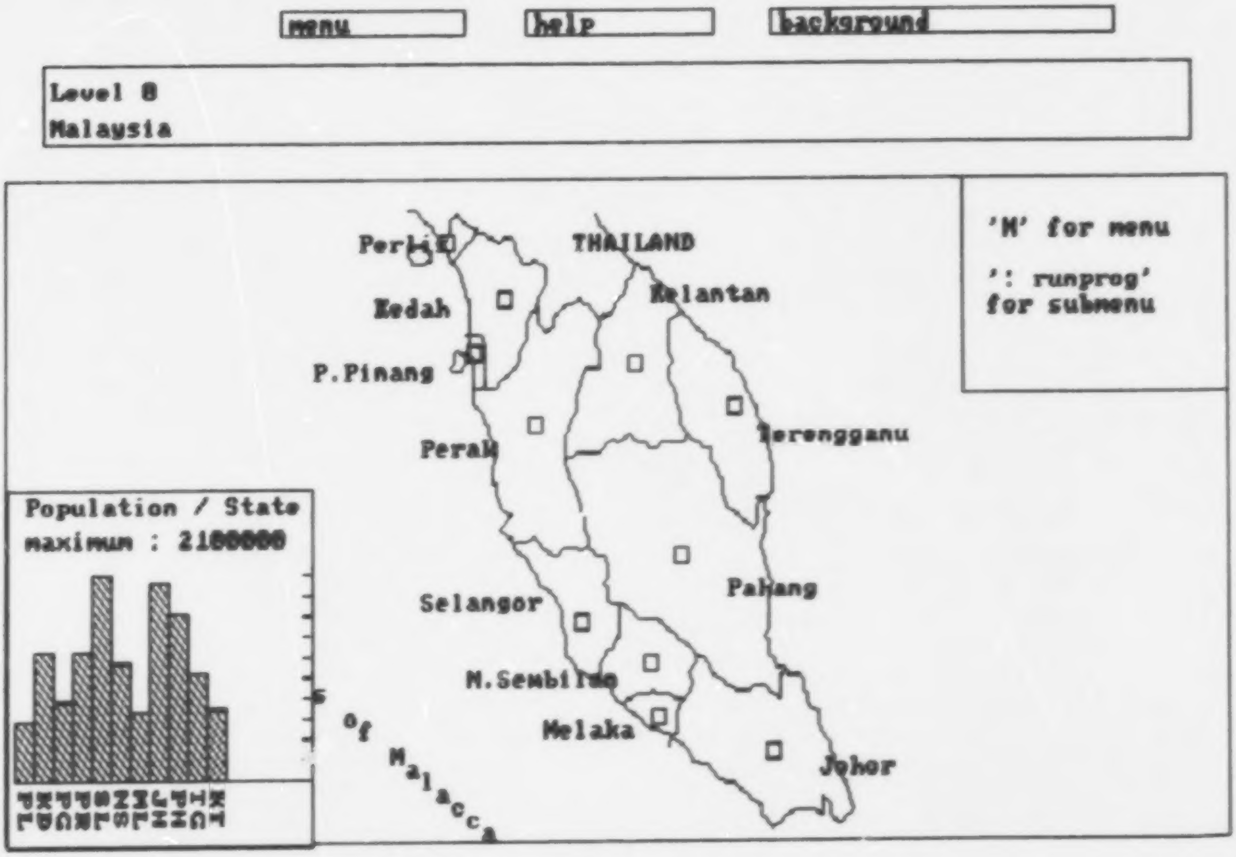


Level 0
Malaysia

'M' for menu

': runprog'
for submenu

Figure 2.

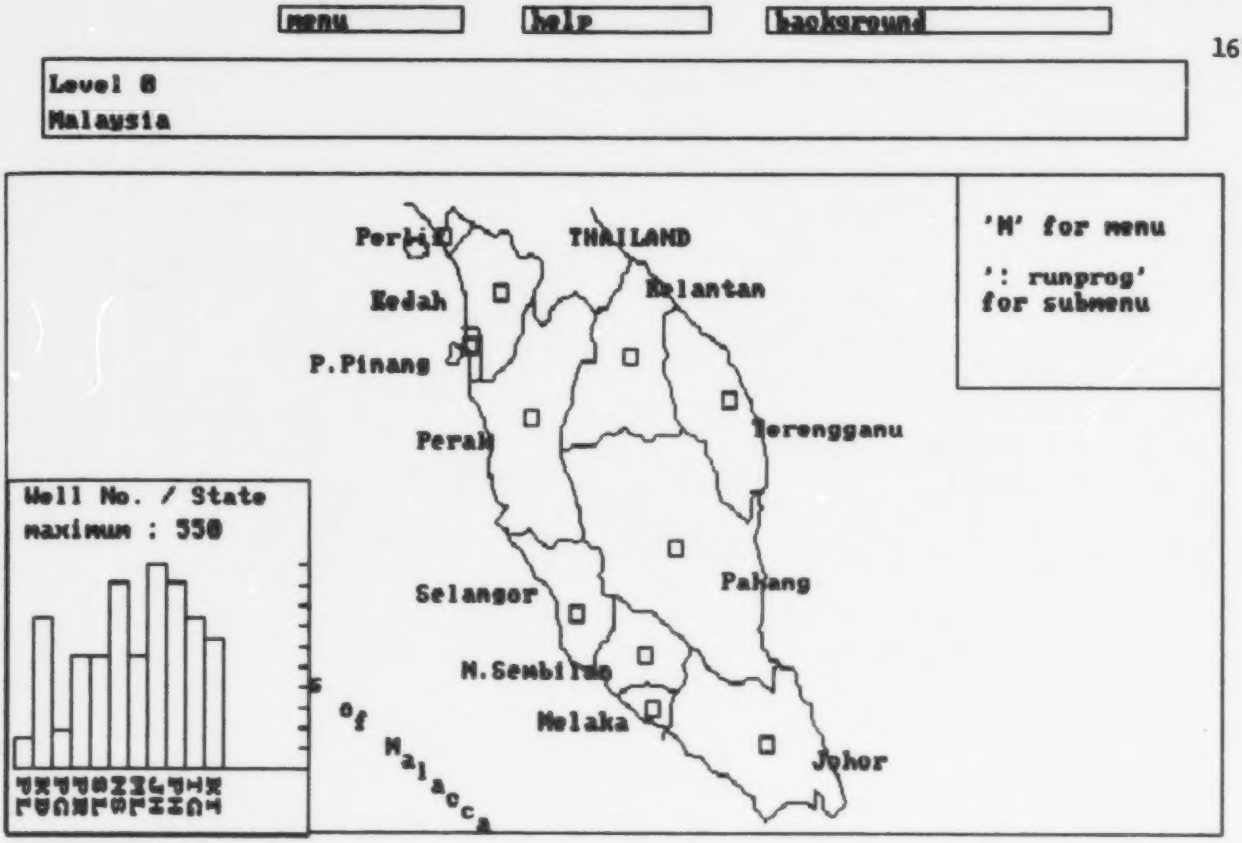


Level 0
Malaysia

'M' for menu

': runprog'
for submenu

Figure 3.

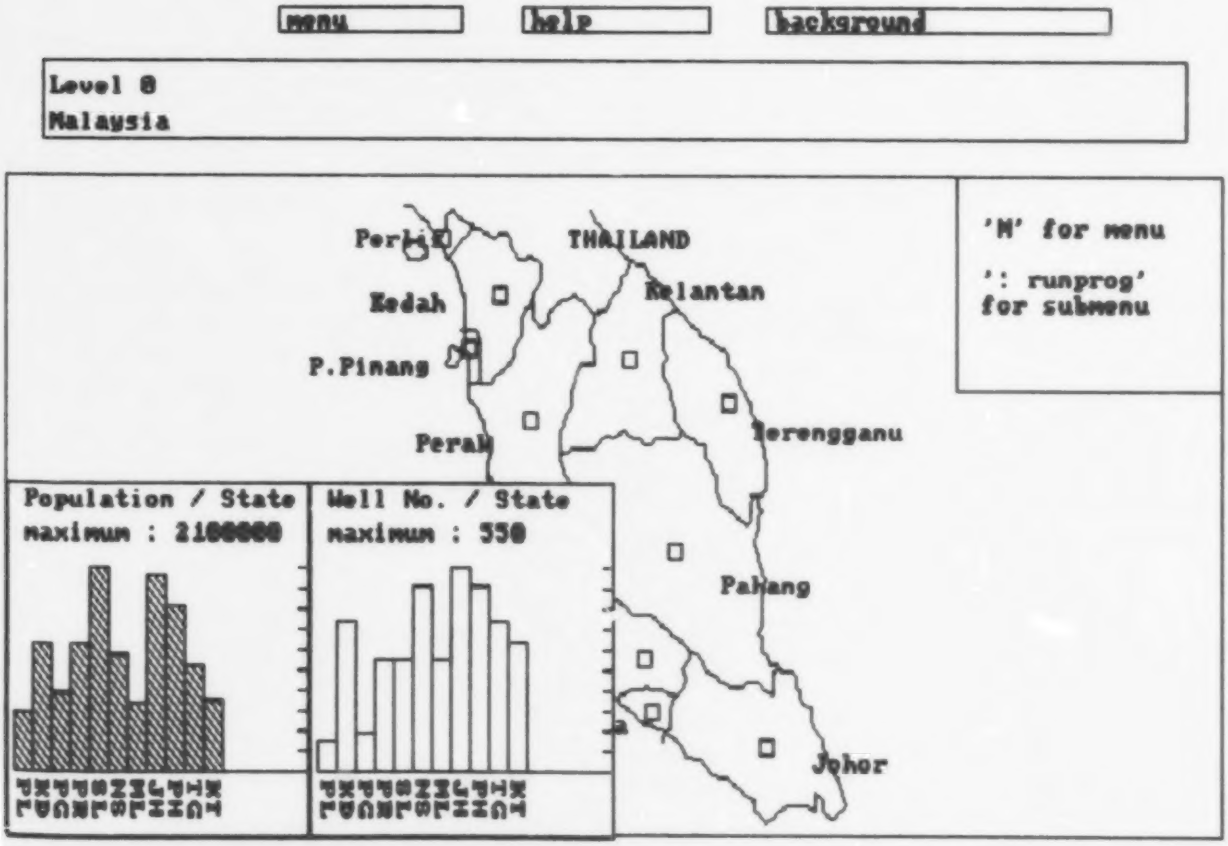


Level 0
Malaysia

'M' for menu

': runprog' for submenu

Figure 4.

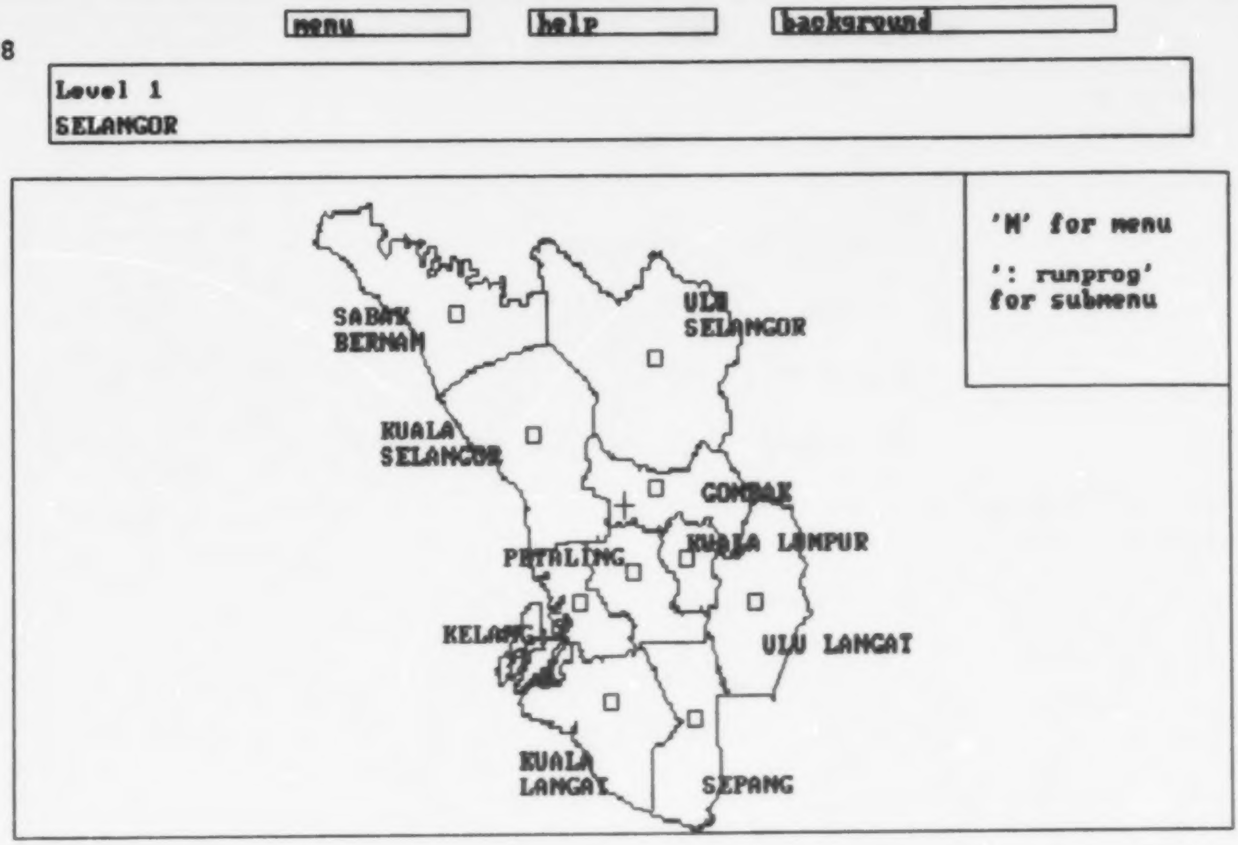


Level 0
Malaysia

'M' for menu

': runprog' for submenu

Figure 5.

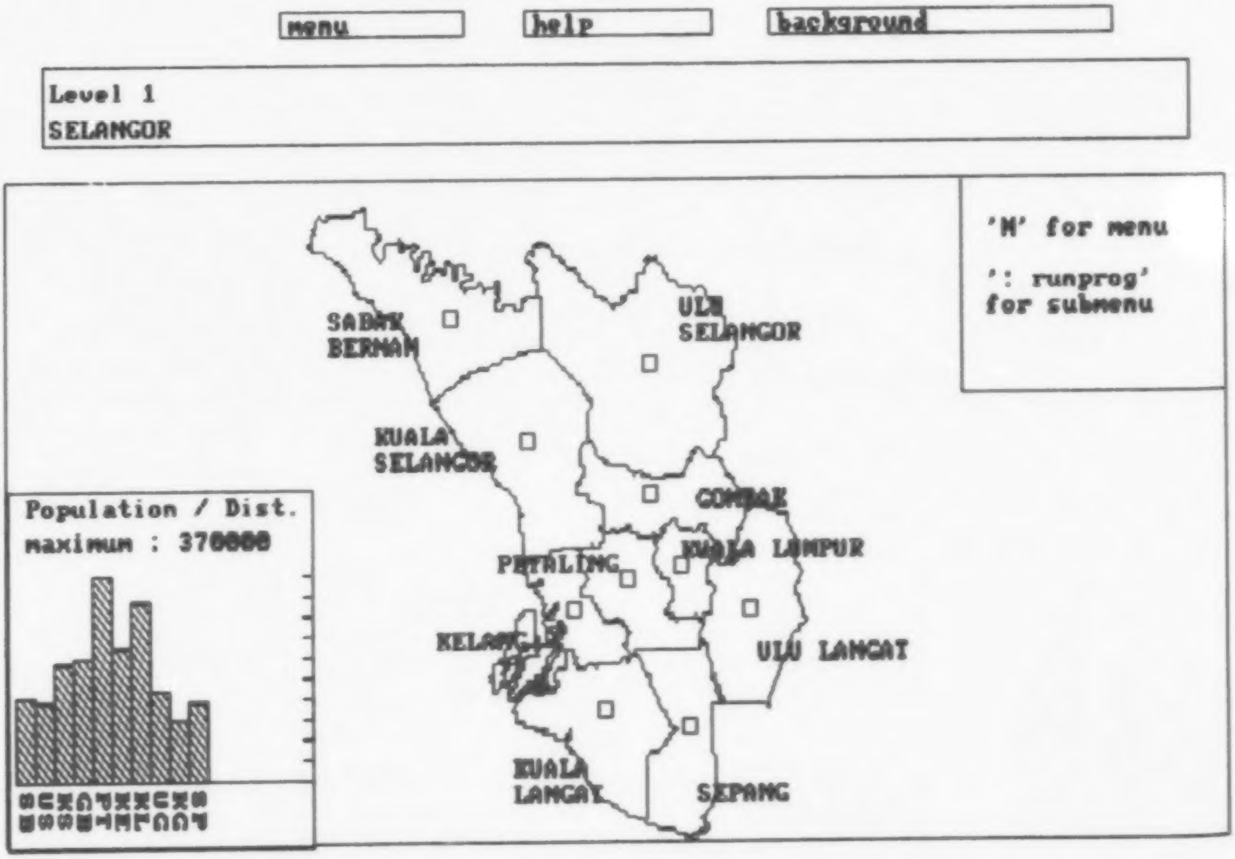


Level 1
SELANGOR

'M' for menu

': runprog' for submenu

Figure 6.

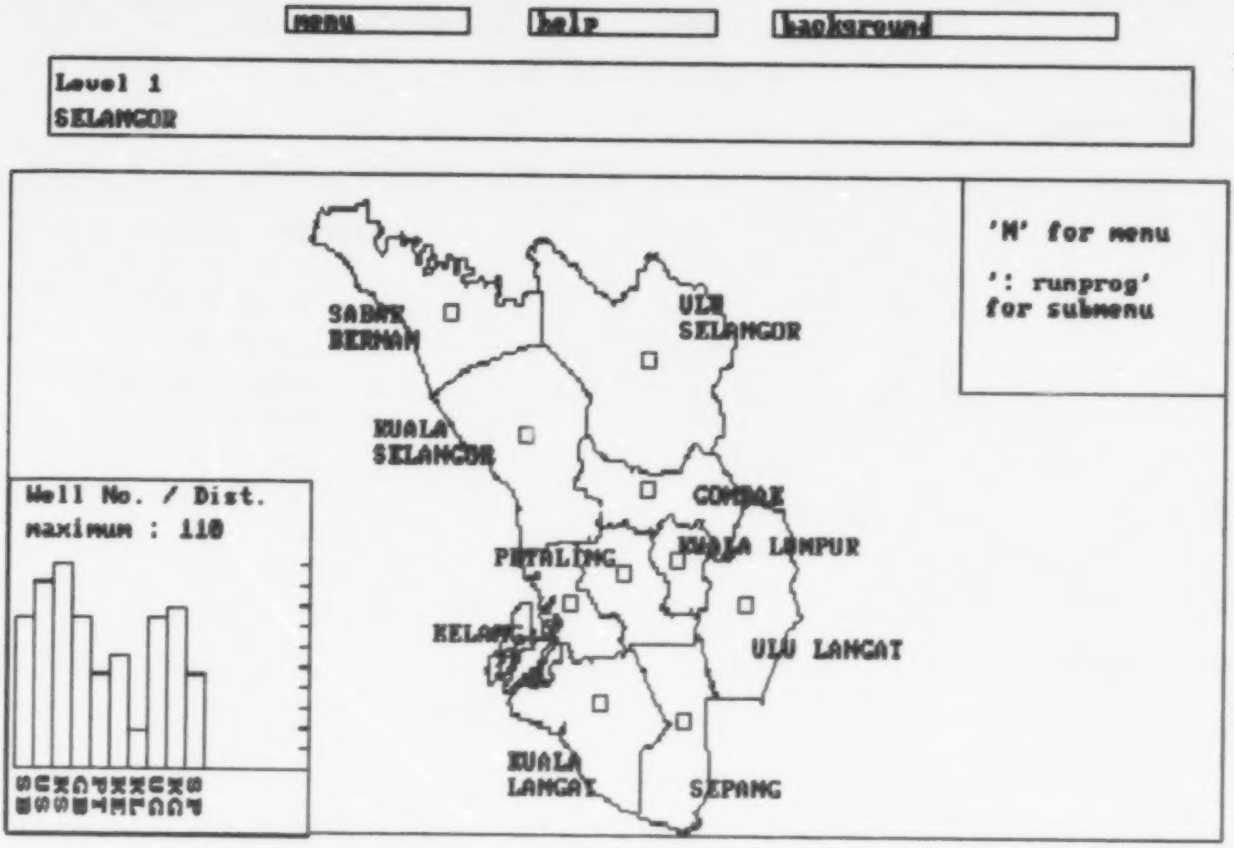


Level 1
SELANGOR

'M' for menu

': runprog' for submenu

Figure 7.



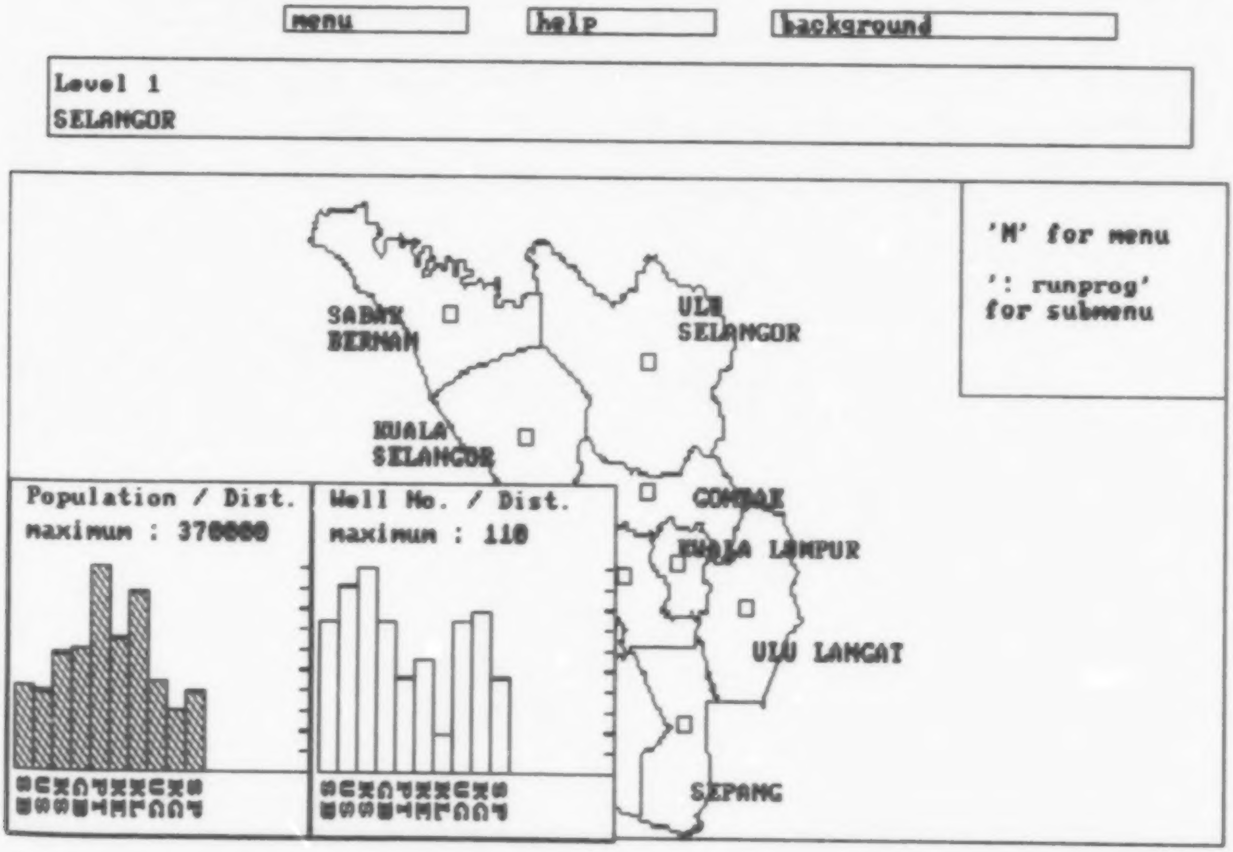
Level 1
SELANGOR

'M' for menu

': runprog'
for submenu

maximum : 110

Figure 8.



Level 1
SELANGOR

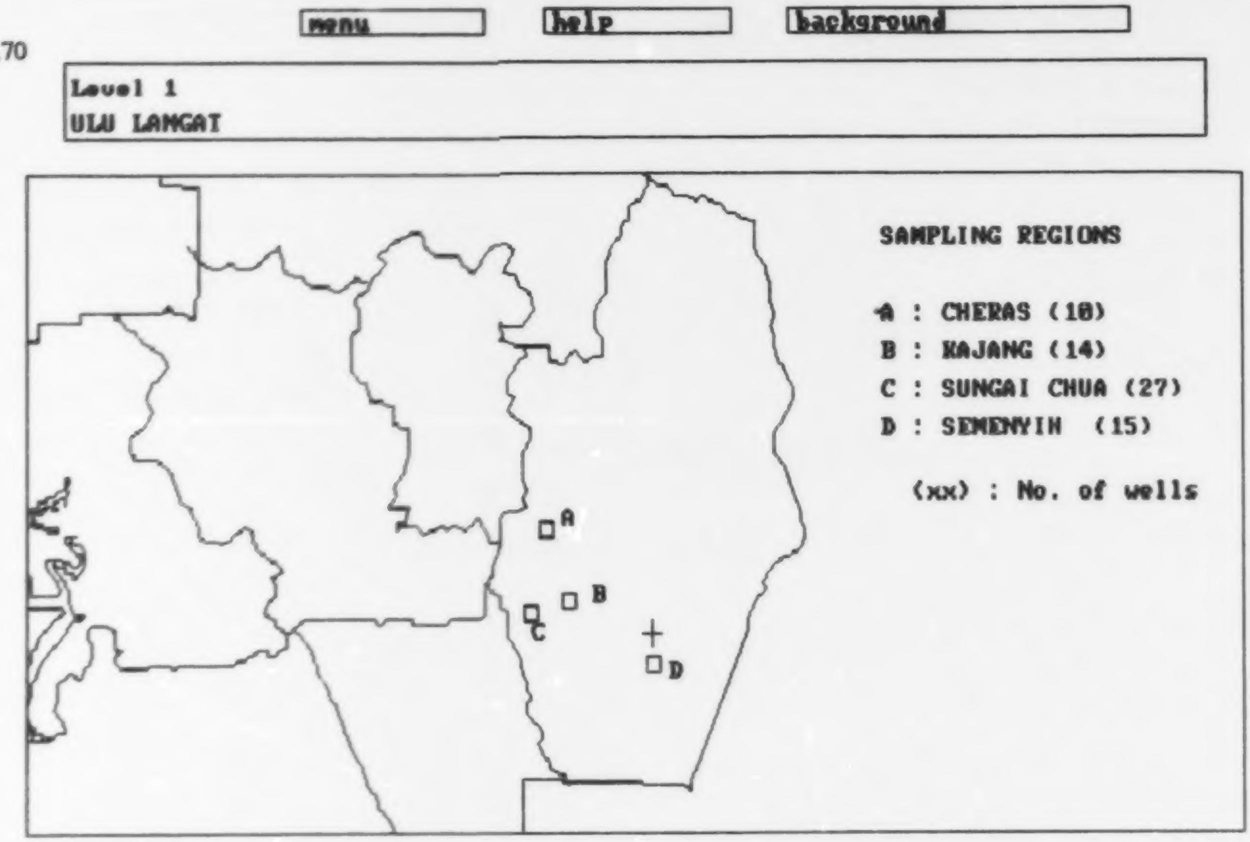
'M' for menu

': runprog'
for submenu

maximum : 370000

maximum : 110

Figure 9.



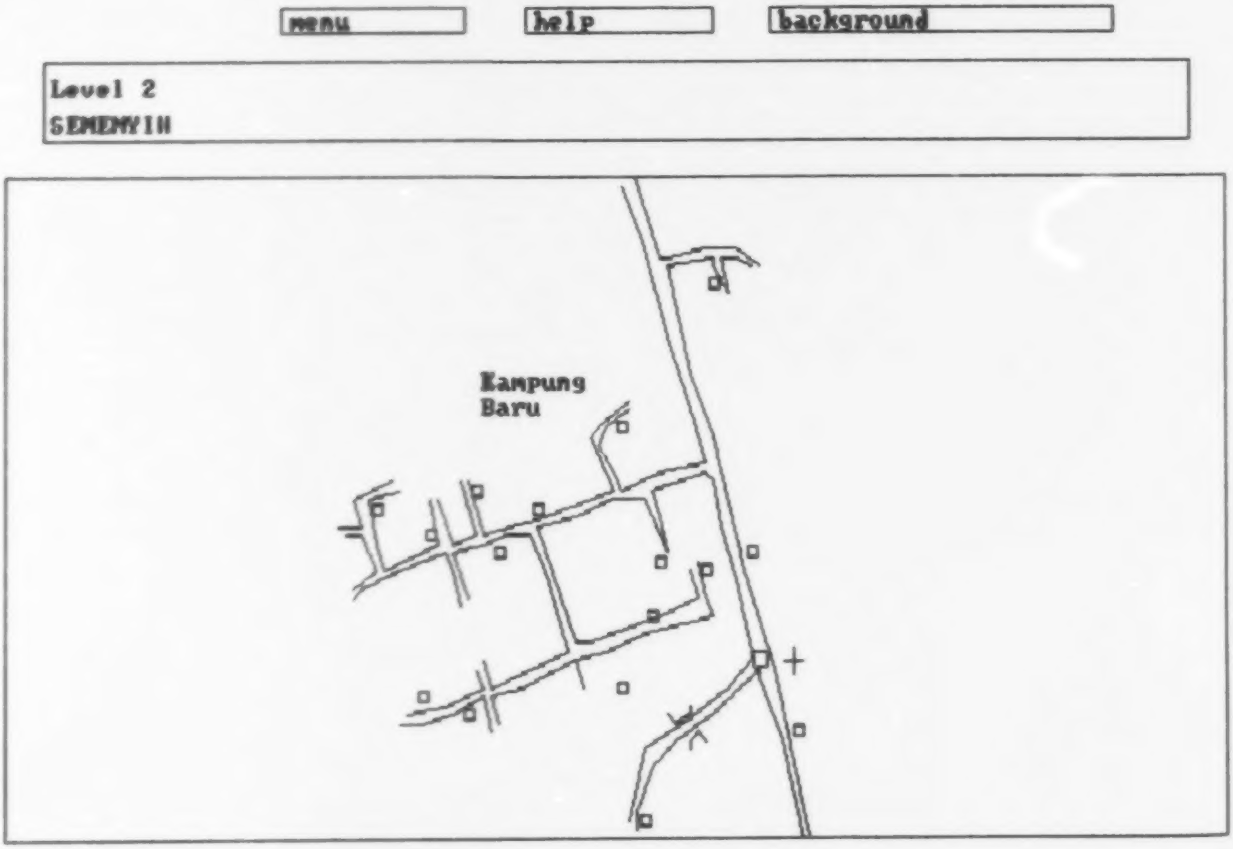
Level 1
ULU LANGAT

SAMPLING REGIONS

A : CHERAS (10)
B : KAJANG (14)
C : SUNGAI CHUA (27)
D : SEMENYIH (15)

(xx) : No. of wells

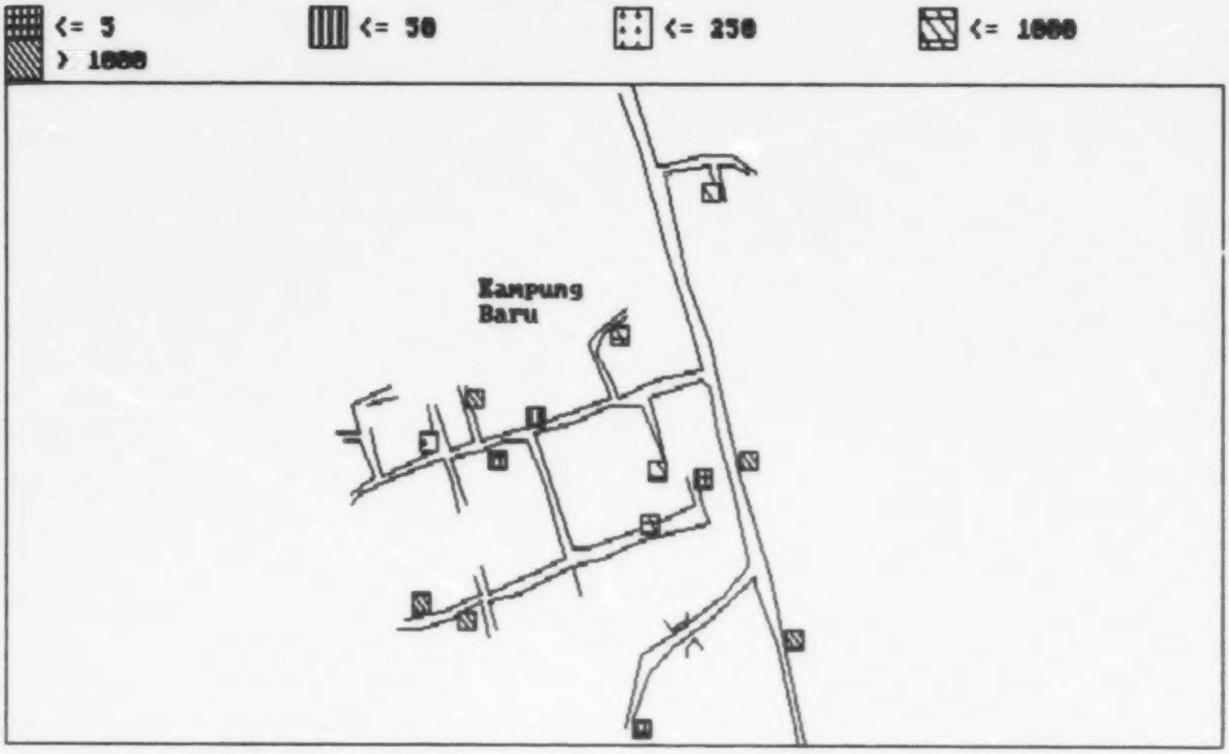
Figure 10.



Level 2
SEMENYIH

Figure 11.

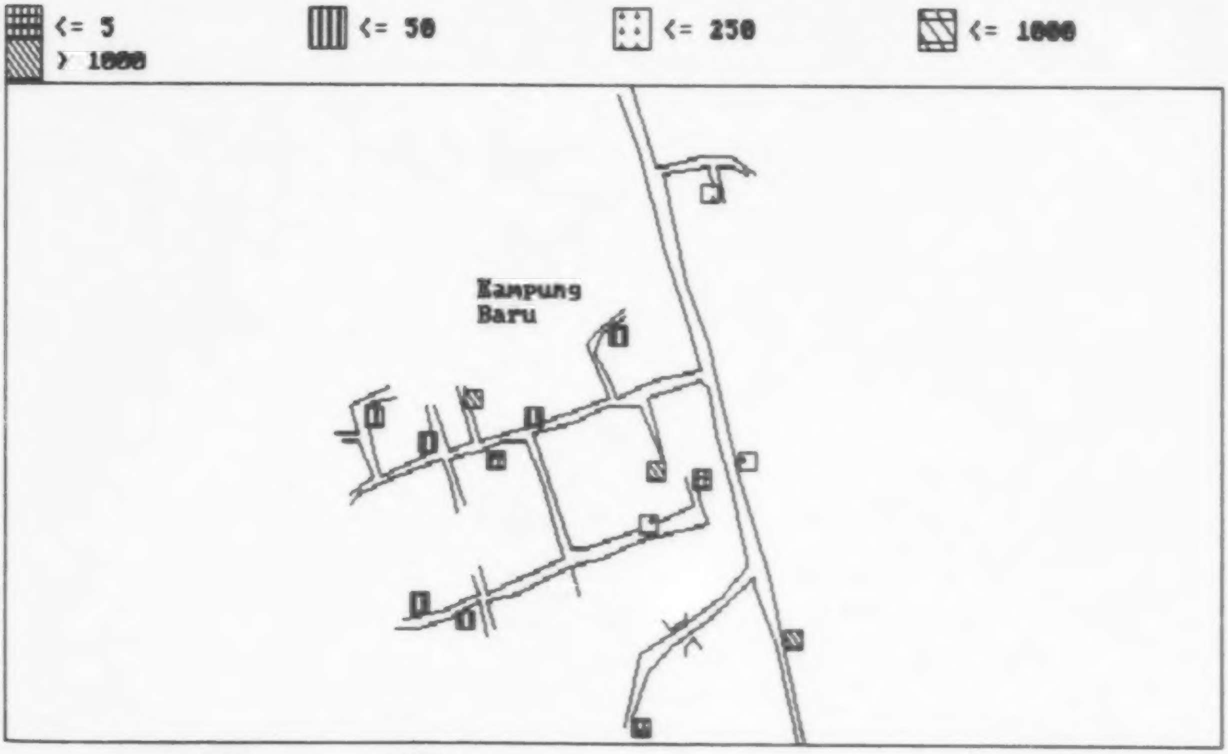
WATER QUALITY FOR SEMENYIH
ULU LANGAT, SELANGOR



YEAR : 88 MONTH : 4 MFC

Figure 12.

WATER QUALITY FOR SEMENYIH
ULU LANGAT, SELANGOR



YEAR : 88 MONTH : 4 COLIPHAGE

Figure 13.

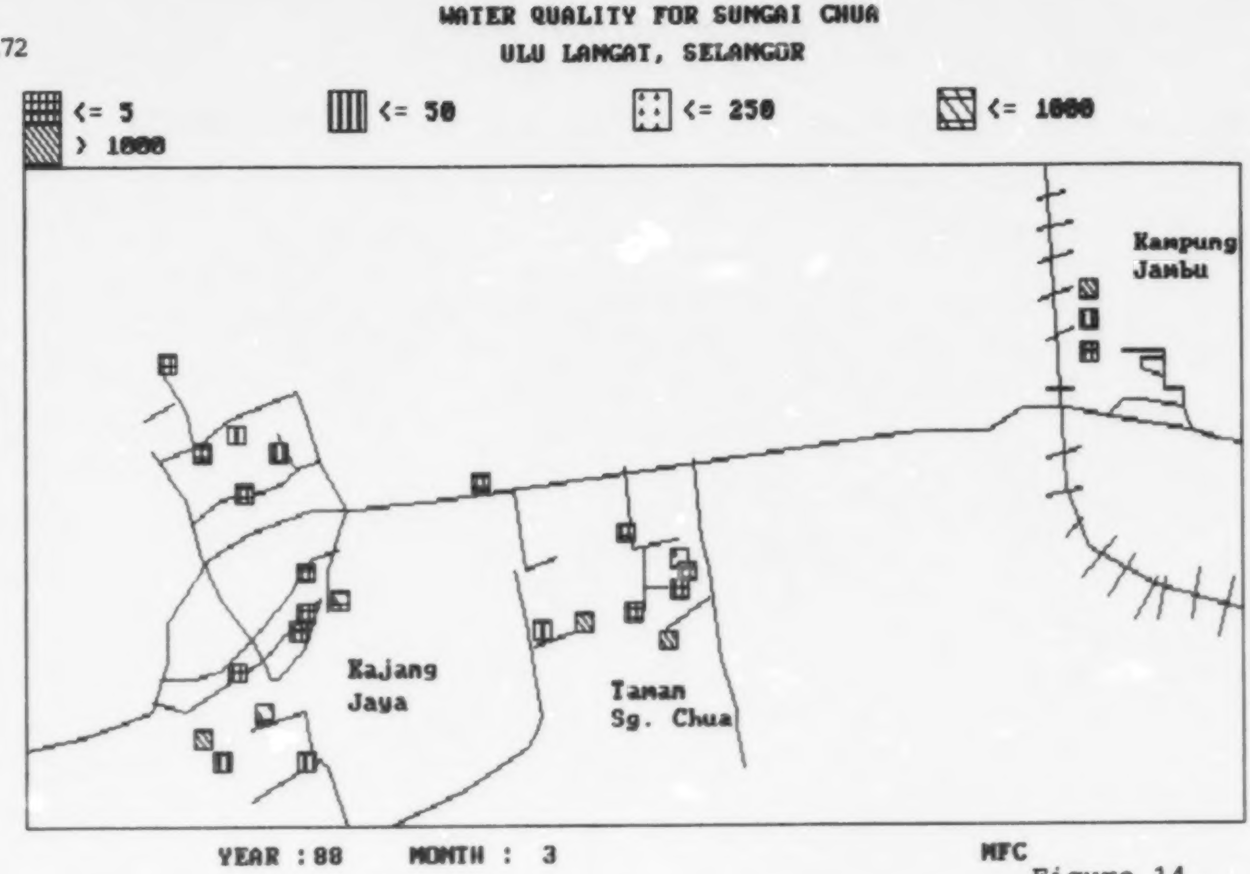


Figure 14.

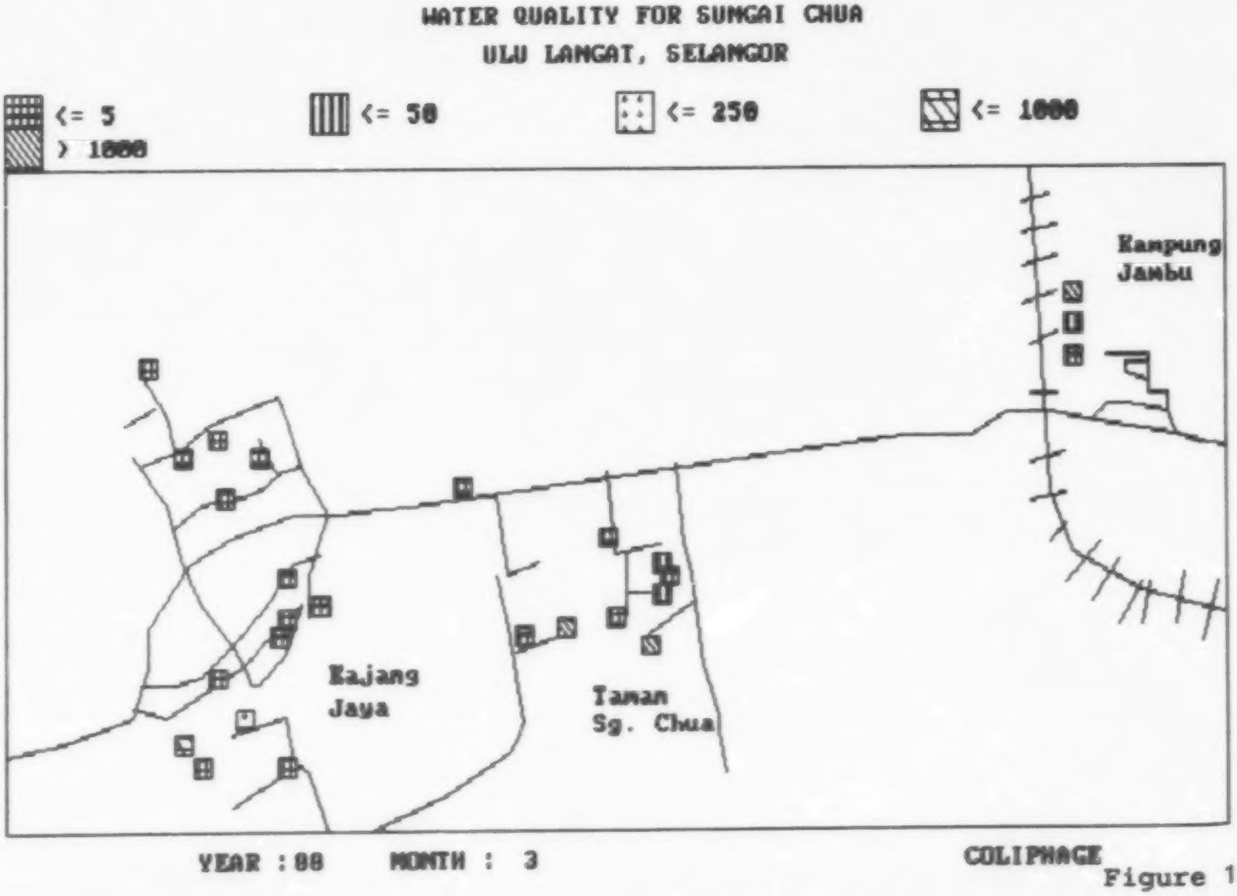
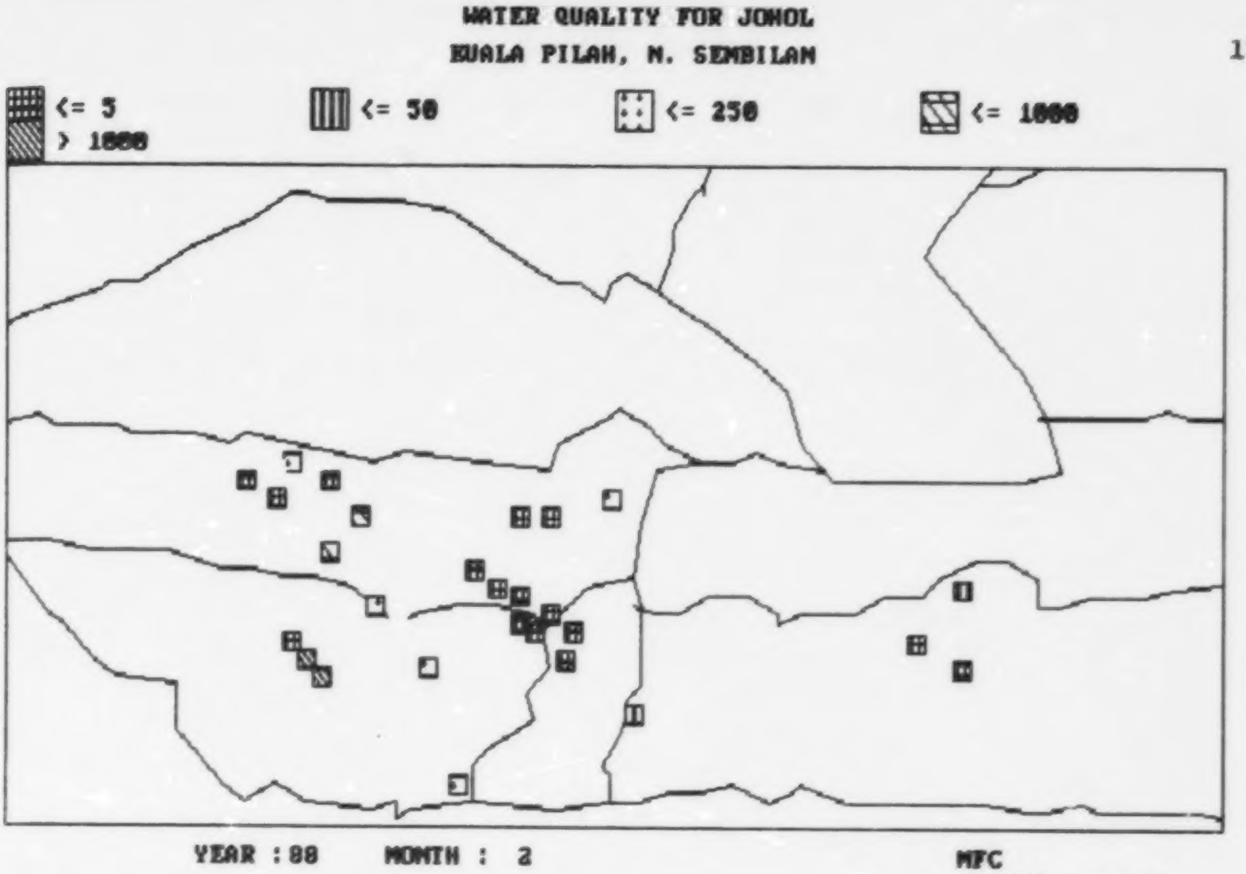
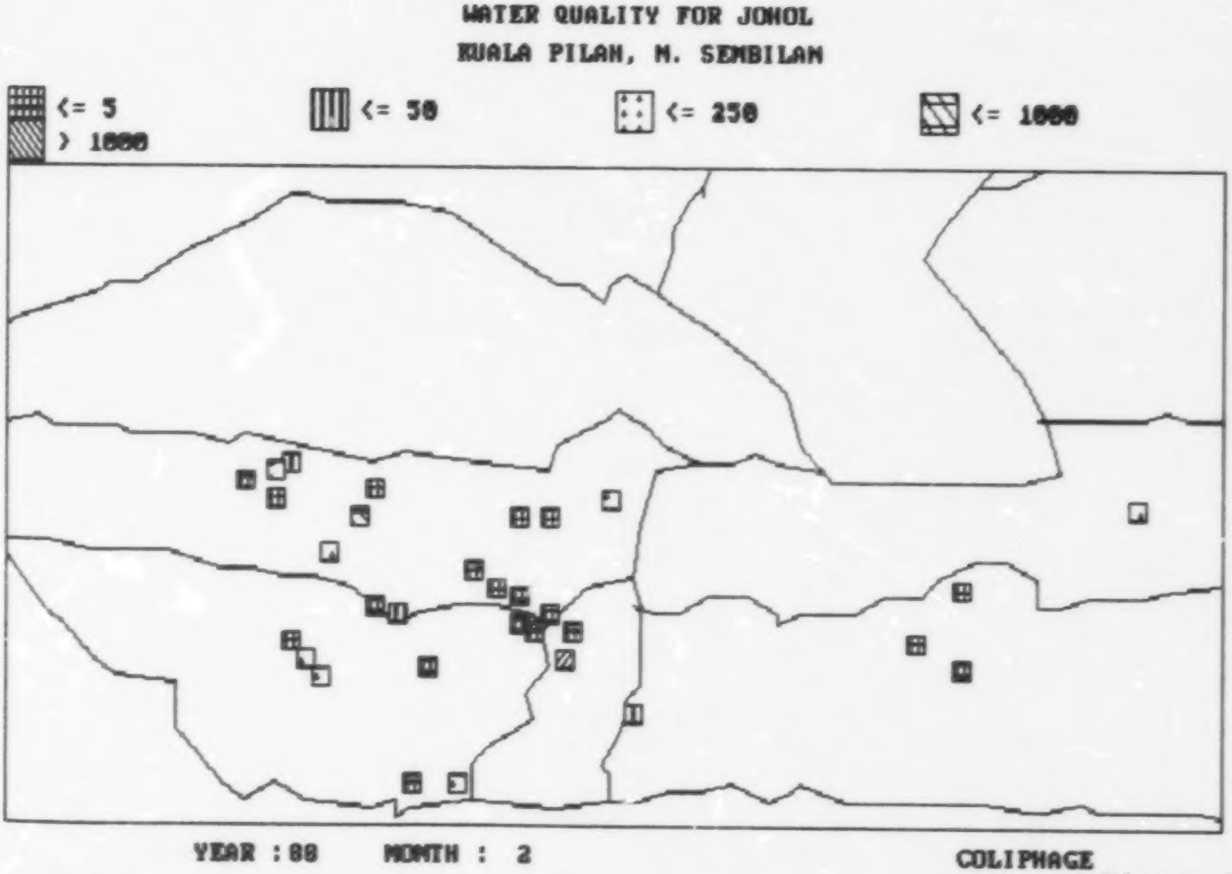


Figure 15.



WATER QUALITY FOR JOHOL
KUALA PILAH, N. SEMBILAN

Figure 16.



WATER QUALITY FOR JOHOL
KUALA PILAH, N. SEMBILAN

Figure 17.

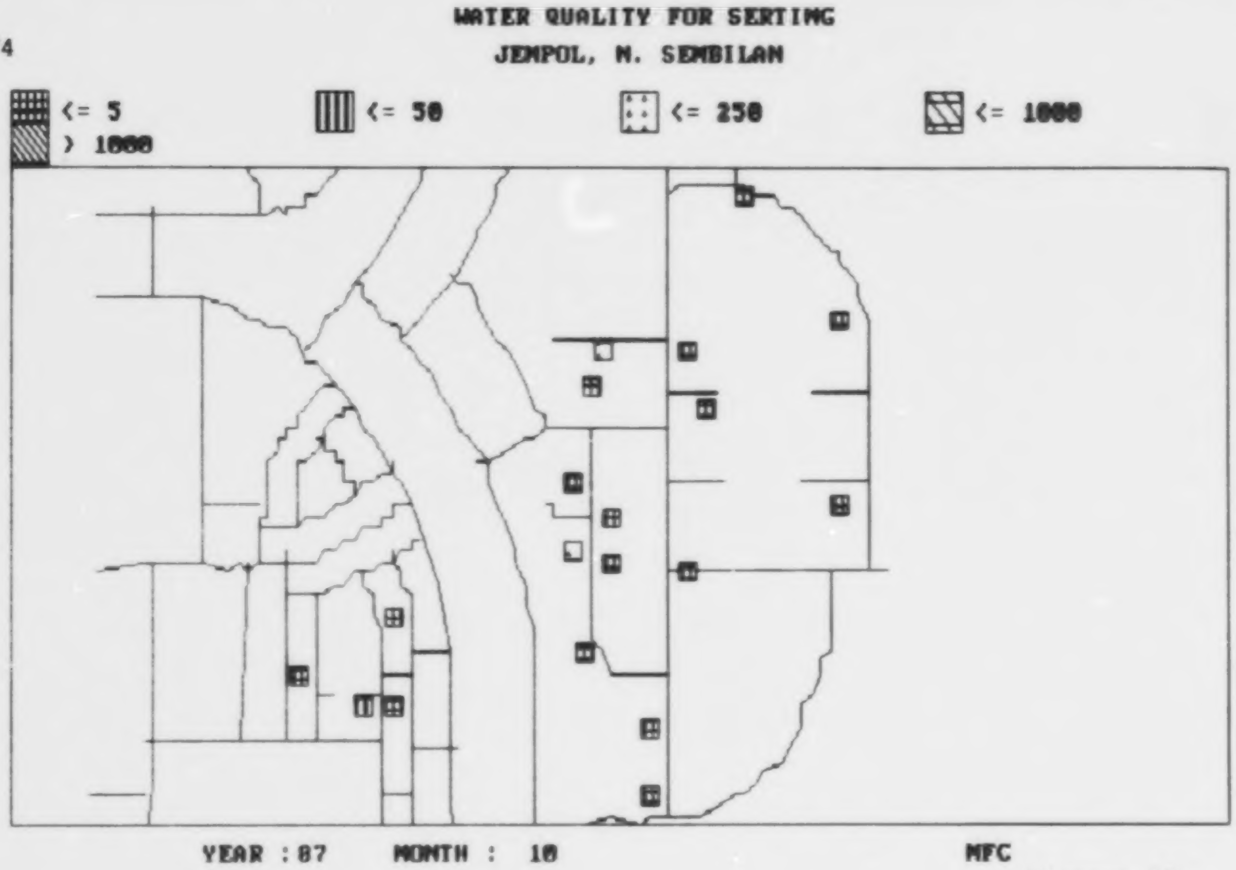


Figure 18.

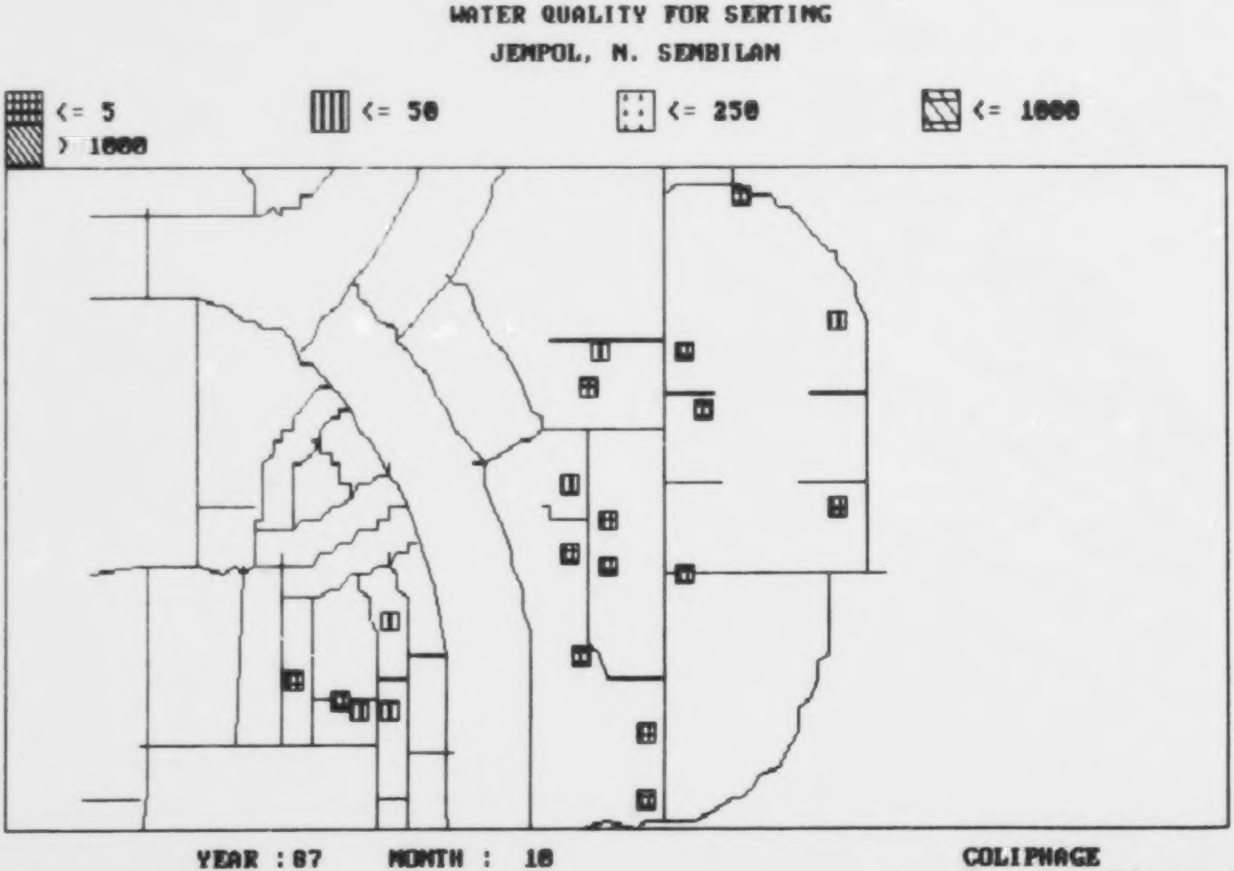


Figure 19.

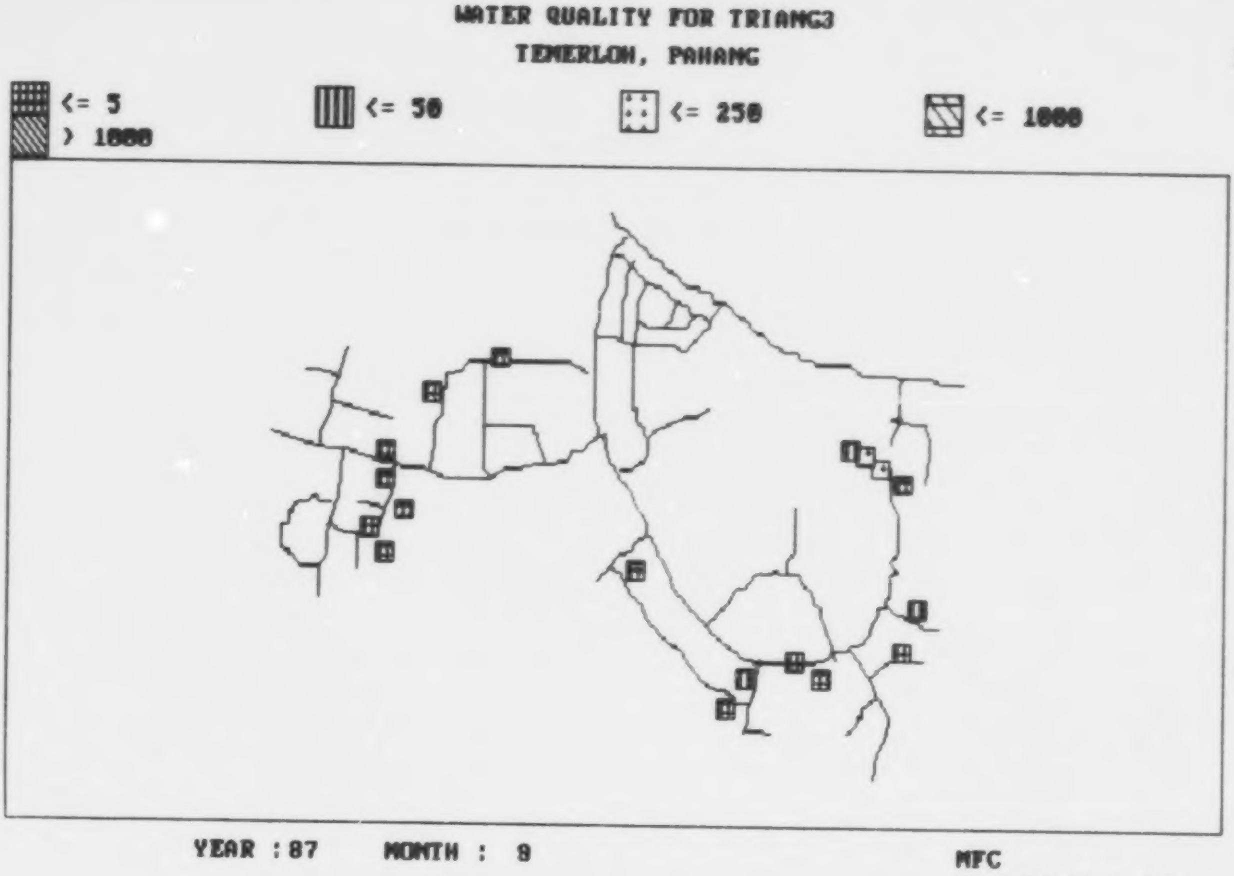


Figure 20.

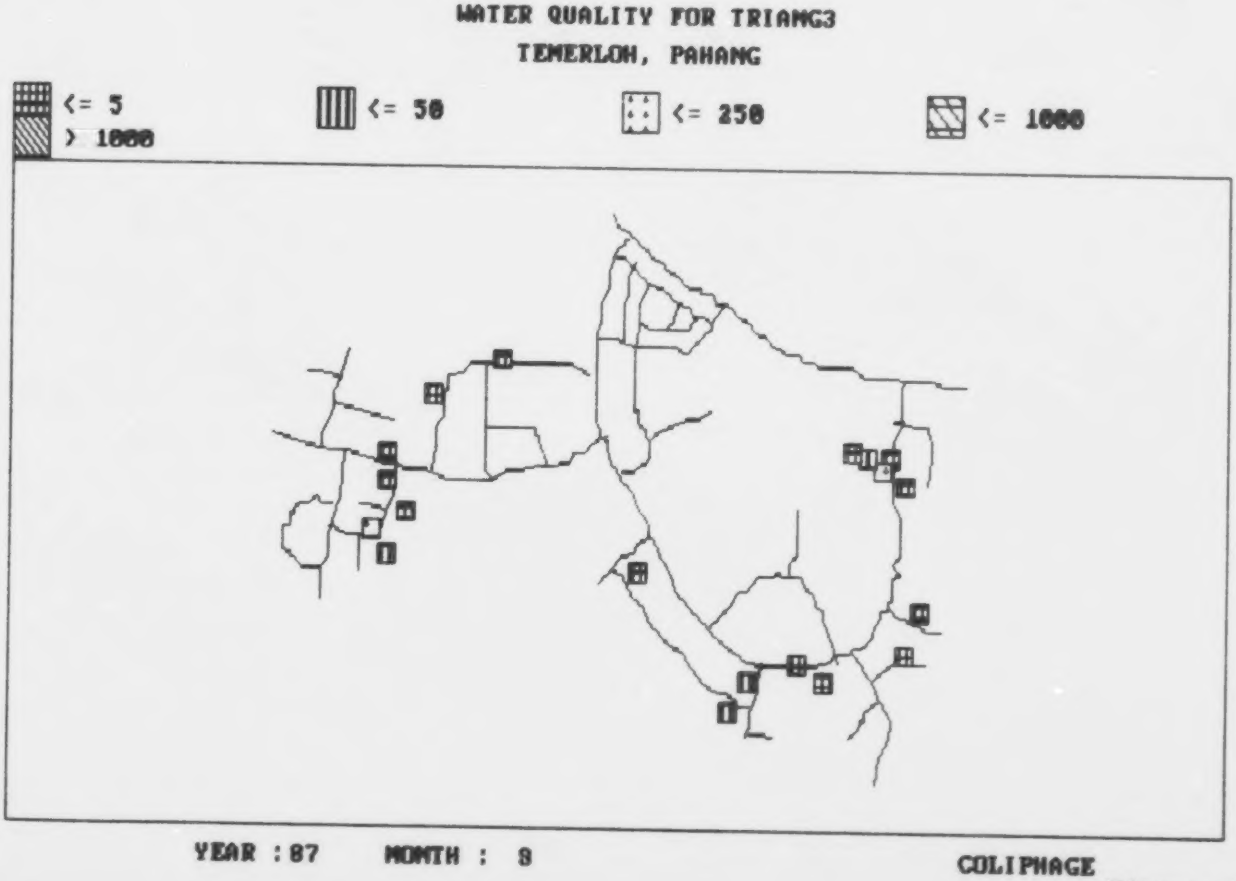
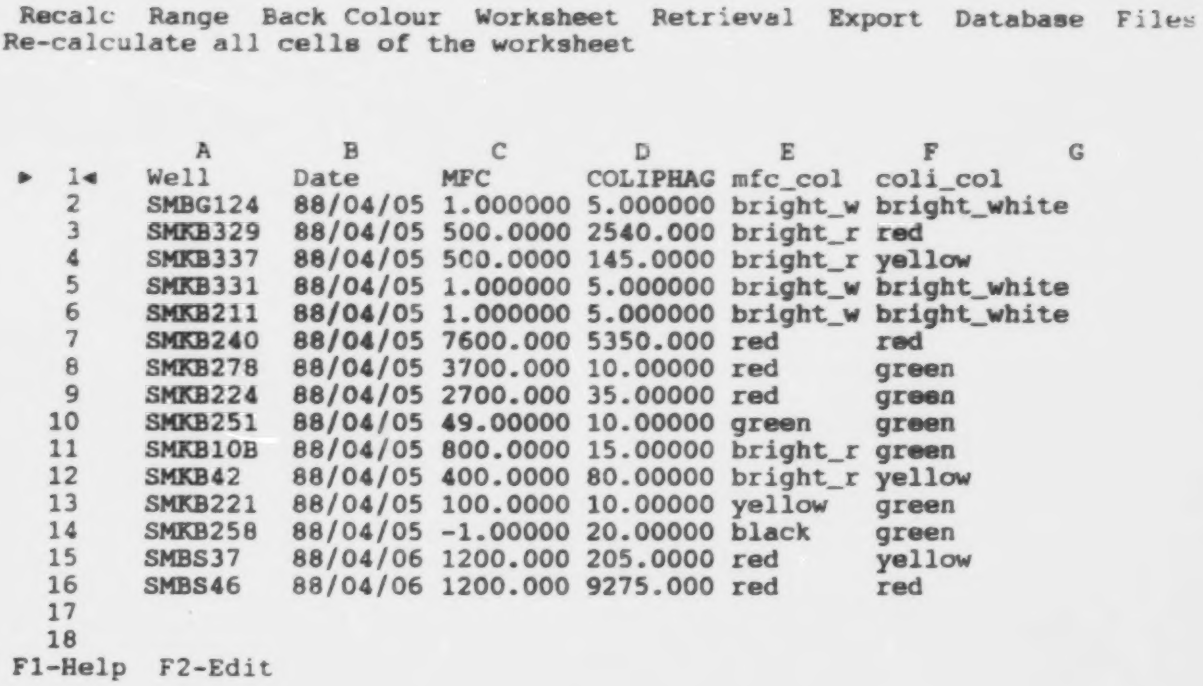


Figure 21.

FIGURE 22

SAMPLE WORKSHEET FOR SOURCE CLASSIFICATION



```
  Recalc  Range  Back Colour  Worksheet  Retrieval  Export  Database  Files
 Re-calculate all cells of the worksheet


                A          B          C          D        E          F          G         H
  ►  1◄    Well       Date       MFC        COLIPHAG  mfc_col    coli_col
     2     SMBG124    88/04/05   1.000000   5.000000  bright_w   bright_white
     3     SMKB329    88/04/05   500.0000   2540.000  bright_r   red
     4     SMKB337    88/04/05   500.0000   145.0000  bright_r   yellow
     5     SMKB331    88/04/05   1.000000   5.000000  bright_w   bright_white
     6     SMKB211    88/04/05   1.000000   5.000000  bright_w   bright_white
     7     SMKB240    88/04/05   7600.000   5350.000  red        red
     8     SMKB278    88/04/05   3700.000   10.00000  red        green
     9     SMKB224    88/04/05   2700.000   35.00000  red        green
    10     SMKB251    88/04/05   49.00000   10.00000  green      green
    11     SMKB10B    88/04/05   800.0000   15.00000  bright_r   green
    12     SMKB42     88/04/05   400.0000   80.00000  bright_r   yellow
    13     SMKB221    88/04/05   100.0000   10.00000  yellow     green
    14     SMKB258    88/04/05   -1.00000   20.00000  black      green
    15     SMBS37     88/04/06   1200.000   205.0000  red        yellow
    16     SMBS46     88/04/06   1200.000   9275.000  red        red
    17
    18
 F1-Help  F2-Edit
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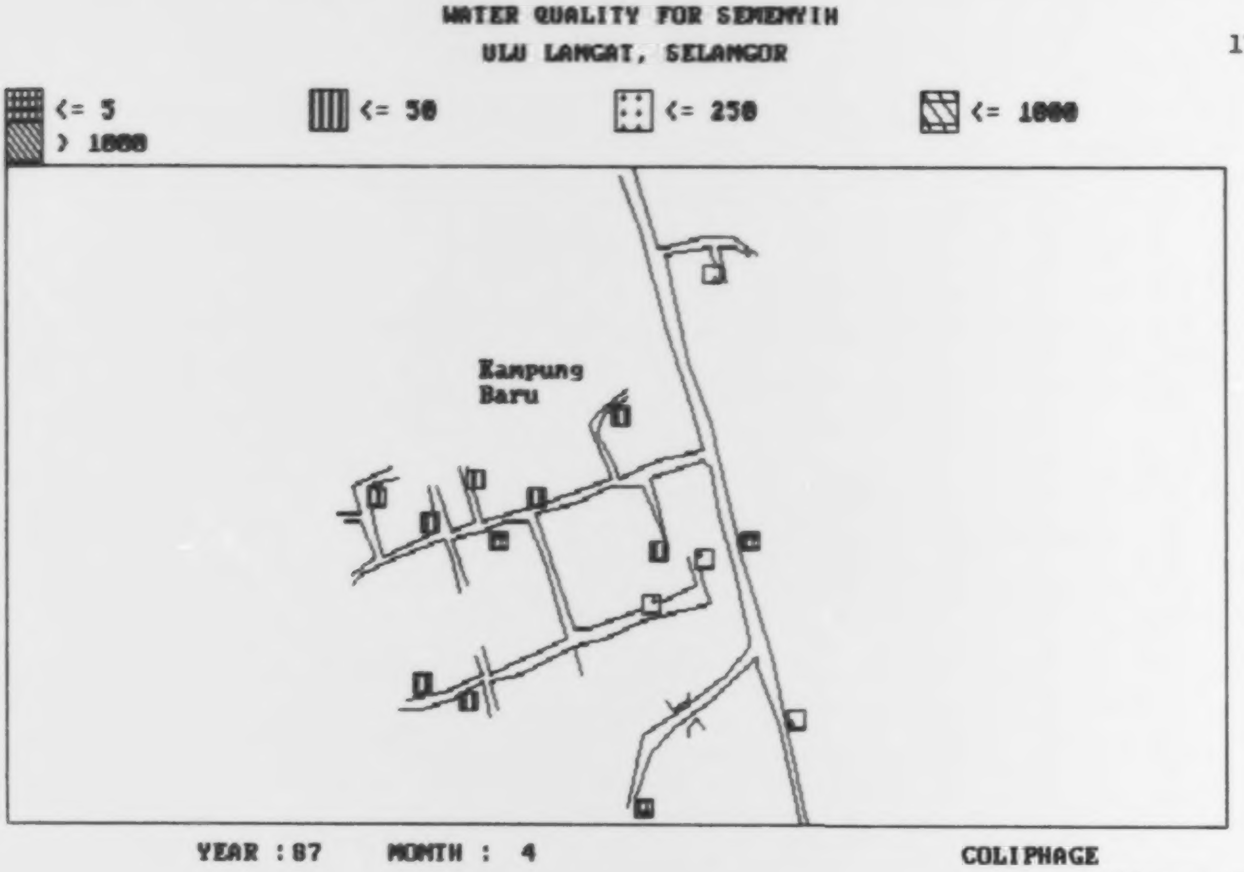

Figure 23.

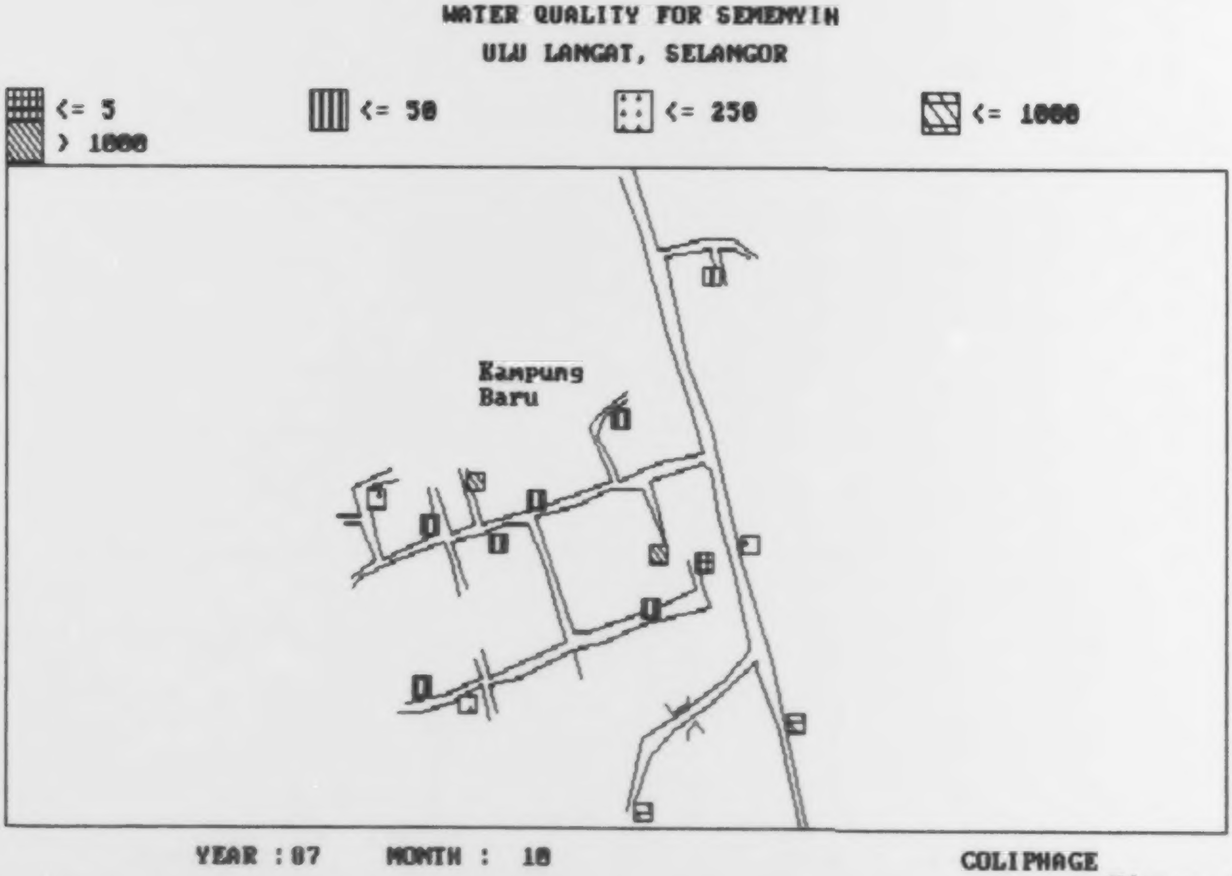

Figure 24.

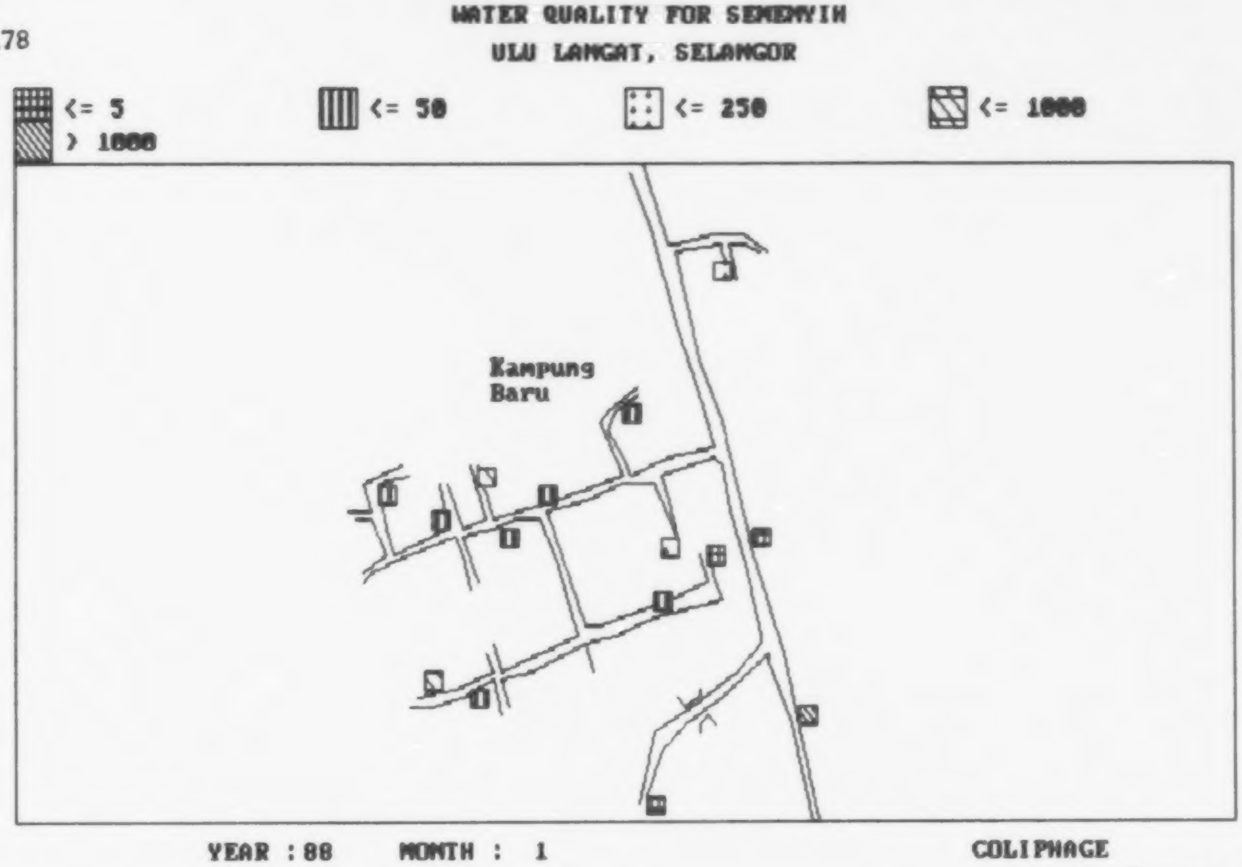

Figure 25.

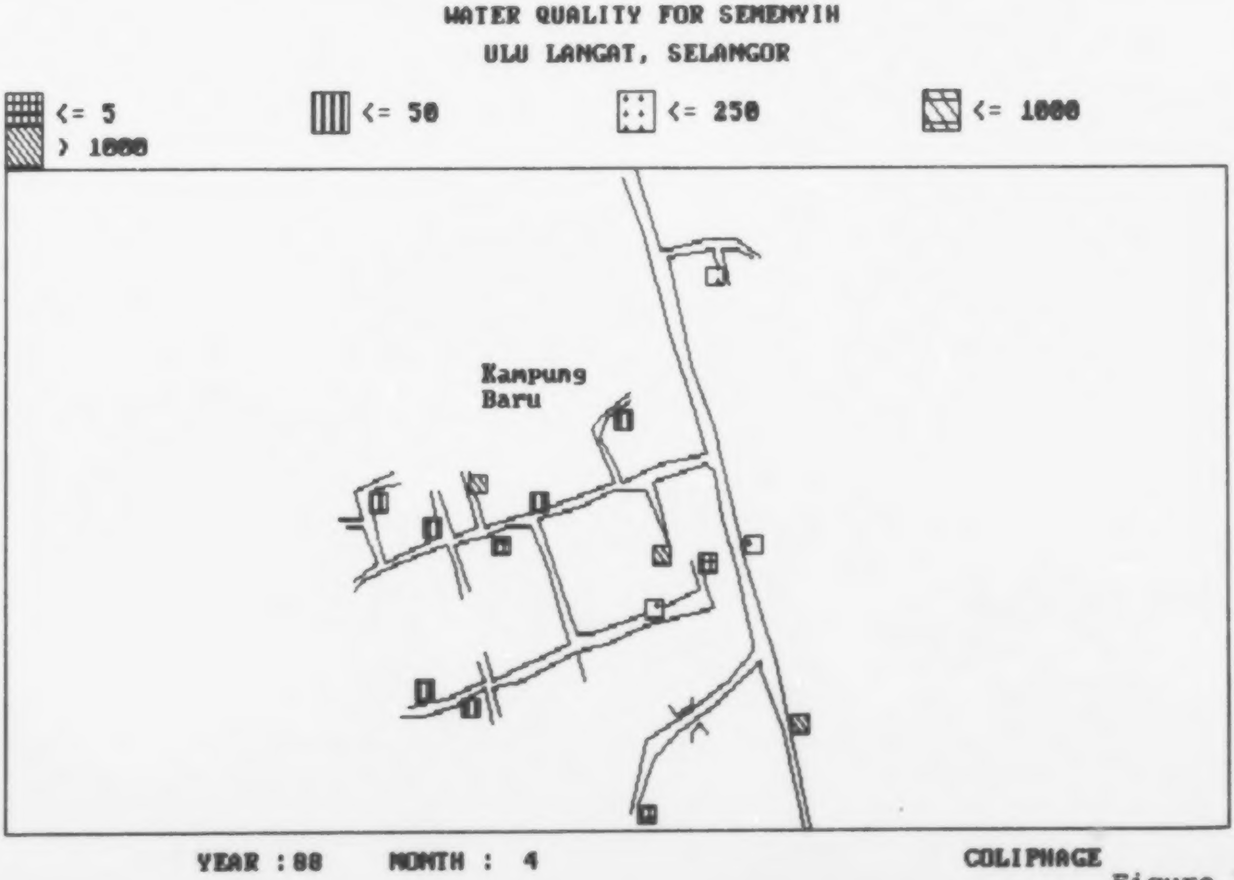

Figure 26.

APPENDIX A

WELL TYPE AND CONDITIONS

WELL : SC931
DATE : 88/03/17
CODE:
RECORD NO. 2

A1. Rain : 3 (6 - No rain since last week
5 - Heavy rain within last week
4 - Rained moderately / lightly the day before
3 - Rained heavily the day before
2 - Rained moderately / lightly when sampling
1 - Rained heavily when sampling)

A2. TYPE of Well (Nab): 399

1 - dug well with bucket system
    (a) Are ropes and buckets permanent* ?
2 - dug well with windlass/motor system
    (a) Is water raising system accessible to user / animals* ?
3 - dug well with pump
    (a) Is priming required* ?
    (b) Was priming carried out when sampling* ?
4 - tube well
    (a) Is priming required* ?
    (b) Was priming carried out when sampling* ?

A3. Depth : 1 (1 - less than 10 m 2 - more 8 - D.K. 9 - N.A.)

A4. Nearest Habitation : 1 (1 - less than 15 m 2 - more 9 - N.A.)

A5. No. of households using : 1 (1 - one
2 - up to 5
3 - up to 10
4 - up to 20
5 - more than 20)

A6. Surrounding Area : 2 (1 - lower level than the well
2 - level
3 - higher level than the well)

A7. Water-logged within 15 m* : 2

* answer the Qs. with 1 - yes 2 - no 8 - D.K. 9 - N.A.

SANITARY PROTECTION

WELL : SC931
DATE : 88/03/17

CODE:

RECORD NO. 2

B1. Protection Condition (abcdef): 213393

(1 - yes and in good condition
2 - no
3 - damage
8 - D.K. 9 - N.A.)

(a) cover
(b) raised parapet
(c) sides sealed
(d) apron
(e) well casing
(f) drainage

B2. Drainage (abcdefghi): 101010001

(a) - within 15 m of water points..............(0/1/8/9)
(b) - earthen channel...........................(0/1/8/9)
(c) - concrete / pipe...........................(0/1/8/9)
(d) - others....................................(0/1/8/9)
(e) - damaged...................................(0/1/8/9)
(f) - clean.....................................(0/1/8/9)
(g) - stagnant..................................(0/1/8/9)
(h) - overflowing...............................(0/1/8/9)
(i) - drain discharge within 15 m..............(0/1/8/9)

B3. Any storage of water* : 1

B4. Any water treatment* : 2

B5. Small Container (abcdefg): 2221211 (0000000 - if large)

(a) Container made from :
  1 - metal
  2 - earthenware
  3 - wood
  4 - plastic
  5 - others
(b) Is there a cover* ?
(c) Where is it kept* ?
  1 - inside the house
  2 - outside the house
(d) Is it exposed to sunlight* ?
(e) How often is the container cleaned ?
  1 - never
  2 - more than once a week
  3 - less than once a week
(f) Is the container clean* ?
(g) Is the water in the container clear* ?

APPENDIX B (continued)

SANITARY PROTECTION

B6. Large Reservoir (abcde): 00000 (00000 - if small)

(a) Reservoir/tank has an inspection manhole*
(b) Inspection manhole protected by a cover or a lock*

(c) Outlets of vents and outflow pipes face downwards*

(d) Vents and outflows pipes protected by grilles*

(e) Rainwater prevented from entering the reservoir/tank*

* answer the Qs. with 1 - yes 2 - no 8 - D.K. 9 - N.A.

POLLUTING SOURCE

WELL : SC931 CODE: RECORD NO. 2
DATE : 88/03/17

C1. Latrine location (abc): 212
(a) 1 - inside 2 - outside the house
(b) 1 - less 2 - more than 15 m
(c) 1 - downhill 2 - level 3 - uphill

C2. Type of Latrine : 6
(1 - flush / pour flush -- jitra
2 - pour flush -- drain
3 - bush
4 - drop
5 - surface / cesspool
6 - trench / borehole
7 - bucket
8 - D.K.
9 - N.A.)

C3. Number of households using latrine : 1
(state how many
97 - more than 96
98 - D.K.
99 - N.A.)

C4. Children using latrine (abcde): 10001

(a) - under 5 years....................(1 / 0)
(b) - defaecate indiscriminately.......(1 / 0)
(c) - into drain.......................(1 / 0)
(d) - into a hole and burried..........(1 / 0)
(e) - use nappies......................(1 / 0)

C5. Disposal of nappies (abcd): 1100

(a) - nappies are used.................(1 / 0)
(b) - washed at water point............(1 / 0)
(c) - washed away from water point.....(1 / 0)
(d) - other methods....................(1 / 0)

C6. Washing and bathing at water point* : 1

C7. Rubbish disposal : 1 (1 - within 15 m 2 - futher 8 - D.K. 9 - N.A.

* answer the Qs. with 1 - yes 2 - no 8 - D.K. 9 - N.A.

APPENDIX D

LAND USE

WELL : SC931 CODE: RECORD NO. 2
DATE : 88/03/17

D1. Type of Soil : 1 (1 - sandy 2 - clay 3 - loam 8 - D.K. 9 - N.A.)

D2. Land Use (abcdef): 000001 (999999 - if none of below)

(a) - agricultural ....(1 / 0)
(b) - denuded.................(1 / 0)
(c) - forest or grassland.....(1 / 0)
(d) - mining area.............(1 / 0)
(e) - swampy..................(1 / 0)
(f) - built-up................(1 / 0)

D3. Agricultural Type (abcd): 0000

(a) - agricultural..............(1 / 0)
(b) - plants ....(1 / 0)
(c) - poultry / livestock ......(1 / 0)
(d) - fish rearing ....(1 / 0)

D4. Manure application* : 2

D5. Animals wander up to the water point* : 1

* answer the Qs. with 1 - yes 2 - no 8 - D.K. 9 - N.A.

RESEARCH NEEDS

During the comparative and investigative studies on the applicability of the coliphage, Presence/Absence (P/A), H_2S paper strip and A-1 broth tests for evaluating water quality, a variety of questions arose. Many of the questions were the result of observed local aberrations with the various tests being evaluated as well as lack of information on the global applicability of these tests.

Summarized below are the research needs which arose out of the research and perceived information gaps.

1. Researchers have noted that coliphage were rarely found in well water. Is this related to the type of pollution, particulate matter or enumeration technique? Research in this area is required.

2. It has been noted that there appears to be no consistent relationship between faecal coliforms and coliphage densities in shallow untreated well waters. Is this related to the type of pollution measurements, e.g. faecal coliform versus total coliforms or the method of estimating both populations, e.g. faecal coliforms - 100 mL by membrane filtration technique or 55.5 mL by MPN techniques while coliphage is estimated by testing 20 mL? Can this relationship be enhanced by using larger volumes of water to test for coliphage content, e.g. an MPN procedure using 5, 10 mL; 5, 1 mL and 5, 0.1 mL portions, or using 10 tubes of 5 mL portions instead of 4 tubes as a routine, or using a 100 mL water sample in large petri dishes?

3. Several researchers have noted that physico-chemical characteristics of the raw and treated waters may be relevant to the coliphage densities found. Research into the effects of such properties as hardness, silt content, particulate content, and disinfection treatment are thought to be important.

4. In some countries the volume of water used in the H_2S paper strip technique varies from 20 to 100 mL. This variation in volume tested may be one of the reasons this procedure works better in one country than in another. Research on the effects of volume and incubation temperature are needed.

5. The majority of research on the reliability and sensitivity of the coliphage, P/A, H_2S paper strip and A-1 broth techniques have been carried out in urban areas, on urban distribution lines and urban wells. There is a need to confirm urban results by evaluating the applicability of those techniques for testing underground waters (deep wells and artesian wells) and rural isolated potable and raw potable

source waters. In these studies comparisons to traditional or local methodologies should be made, including cost benefits.

6. Since the majority of the developing countries do not have the means to develop an inventory of their aquatic resources nor an economically viable scheme to prioritize them according to the quality of water they contain for drinking water purposes, research into the feasibility of using either or both the coliphage and A-1 broth techniques to evaluate and prioritize these surface and subsurface waters should be initiated.

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